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By

**P. Herbert Carpenter, M.A.,**  
Assistant Master at Eton College.

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With Plate I.

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IN a previous volume of this Journal<sup>1</sup> I have discussed the bearings of Ludwig's researches into the early development of the skeleton in Starfishes and Ophiurids on his views respecting the homologies of these two types of Stellerids. Further consideration of this question, especially with respect to the Ophiurid-skeleton, has led me to results of some interest, which I propose to explain in the following pages. I should wish, however, first of all, in justice to my friend Ludwig, to correct an error which unfortunately found its way into my last paper. He is represented, on p. 385, as still believing the so-called "odontophore" of Asterids to be homologous with the mouth-shields of Ophiurids. This is a view which he expressed in his earlier papers<sup>2</sup>; and whatever its intrinsic merits, it is altogether untenable if the mouth-shields of Ophiurids represent the so-called "genitals" of Asterids, on account of their mutual relationship to the water-pore, as Ludwig has since

<sup>1</sup> Vol. xxii, new series, October, 1882, pp. 371—386.

<sup>2</sup> 'Morphologische Studien an Echinodermen,' Band i, "Zur Kenntniss der Gattung Brisinga," pp. 231, 235. "Beiträge zur Anatomie der Ophiuren," pp. 263, 269, Band ii. "Das Mundskelett der Asterien und Ophiuren," p. 3. The author has unfortunately omitted the word "früher" on line 6 of this page, which should read "die früher von mir angenommene Homologie." Compare p. 16.

suggested.<sup>1</sup> I ought, however, to have remembered that in his last-published paper, describing the development of *Asterina gibbosa*, he had definitely (though for the first time) withdrawn his earlier suggestion; for I had specially marked the passage in the copy of his memoir which he was good enough to send me. It is as follows:<sup>2</sup>

“Im Gegensatze zu meiner früheren Auffassung, wonach dieses Skelettstück (i. e. Odontophor) als die erste intermediäre Skelettplatte bezeichnet werden müsste, bin ich jetzt zu der Ansicht gelangt, das dasselbe mit den vorhin besprochenen paarigen Interambulacralplatten in einer Reihe gehöre und demnach als unpaare Interambulacralplatte aufzufassen sei.”

Ludwig, therefore, no longer considers the odontophore of an Asterid as homologous with the mouth-shield of an Ophiurid and oral of a Crinoid. I am by no means sure, however, that his earlier view is not the more correct one after all; but I am quite content to leave this question in the hands of my friend Mr. Sladen, whose opportunities of studying the modifications of the Asterid skeleton have been unrivalled.

One very important result of his observations is fully explained by himself in the following paper (pp. 29—34), and I will do no more than just refer to it. Ludwig's discovery of the true nature of the terminal plates in the Ophiurid arms<sup>3</sup> will be remembered by readers of my fifth note.<sup>4</sup> The terminals (Pl. I, figs. 12, 13 τ) are not “radials” homologous with the ocular plates of an Urchin, as has been generally supposed since the time of Müller; for, so far as we yet know, the representatives of these plates remain on the disc, sometimes close to the dorsocentral, and sometimes separated from it by intervening plates (Pl. I, figs. 1—4, 6—9; 4). They

<sup>1</sup> ‘Morph. Stud.,’ ii, loc. cit., p. 16. “Ueber den primären Steinkanal der Crinoideen, nebst vergleichend-anatomischen Bemerkungen über die Echinodermen überhaupt,” pp. 42, 43. “Neue Beiträge zur Anatomie der Ophiuren,” pp. 66, 79. “Entwicklungsgeschichte der *Asterina gibbosa*,” p. 181.

<sup>2</sup> ‘Morph. Stud.,’ ii, p. 181.

<sup>3</sup> ‘Morph. Stud.,’ ii, “Zur Entwicklungsgeschichte des Ophiurenskelettes,” pp. 97, 98.

<sup>4</sup> This Journal, vol. xxii, new series, October, 1882, pp. 379, 380.



are the plates which are generally called "primaries" in specific descriptions, and their real nature has only lately become apparent. The terminals, on the other hand, are an additional set of plates altogether, which are carried outwards from the disc at the ends of the growing arms (Pl. I, figs. 12, 13;  $\tau$ ).

In describing the young Asterid, however, Ludwig followed his distinguished predecessors Lovén and A. Agassiz, and spoke of the terminal plates as radials.<sup>1</sup> A difference was thus established between the Asterids and Ophiurids respectively, which could not easily be explained, affecting as it would not merely the fate of the radial abactinal plates, but also the nature of the arms. In a later phase of Asterid development<sup>2</sup> Ludwig found five radially situated plates (Pl. I, fig. 15; 4) occupying an intermediate position between the terminals and the primary interradials (genitals); and he spoke of them as the forerunners of a system of intermediate plates which form the greater part of the dorsal skeleton of the disc and rays. In the diagram<sup>3</sup> which I gave of the apical system of the larval Asterid I followed Ludwig's nomenclature; but Sladen now suggests that these plates (*in* of the diagrams given by Ludwig and myself) are the true radials. They would thus be homologous with those of an Ophiurid and Crinoid, and should therefore have been marked 4 in my diagram, as they are in Sladen's revised edition of it (Pl. I, fig. 15); while the plates marked 4 in my figure, and called radials, or preferably "Terminalia" by Ludwig, are really terminals corresponding to those of Ophiurids, and should have been lettered  $\tau$ , as in fig. 1 on p. 379 of my fifth note (Pl. I, fig. 13). Sladen's reasons for this important suggestion are set forth in his own paper a few pages farther on; but I wish to say that I entirely acquiesce in it, as also in his recognition of the presence in Asterids of plates homologous with the

<sup>1</sup> 'Morph. Stud.,' ii, p. 160.

<sup>2</sup> Ibid., p. 180.

<sup>3</sup> This Journal, vol. xxii, 1882, p. 382, fig. 4. This is reproduced with slightly different lettering on Pl. I, fig. 15.

under-basals of Crinoids.<sup>1</sup> The uniformity in the composition of the apical system or calyx in all the three groups of brachiate Echinoderms thus becomes exceedingly striking.

It will be remembered that I have pointed out the presence of under-basals in the young *Amphiura squamata*<sup>2</sup> (Pl. I, fig. 12; 2). Since then I have searched through all the figures of the disc in adult Ophiurids which I could find, especially those in Lyman's admirable memoirs; and I have been surprised to discover in what a number of species the homologues of the plates of the Crinoidal calyx may be detected in the rosette which occupies the centre of the disc.

Lyman<sup>3</sup> describes this rosette as made up of "primary plates which lie on the back of the disk, one in the centre, and ten others radiating from it; they may be distinguished by their greater size. In the young animal they cover the whole back of the disc and are only six in number." He describes these six elsewhere,<sup>4</sup> as arranged with "the largest in the centre and the other five in a close circle round it, one opposite the base of each arm," i.e. the "primary" plates of the young Ophiurid are radial in position. This is well shown in the larval *Amphiura squamata* described by Ludwig. A diagram based upon one of his figures appeared in my fifth note and is reproduced in Pl. I, fig. 13. The same point is also well illustrated by two figures of the young *Hemipholis cordifera* given by Lyman.<sup>5</sup> At a later stage of development one or even two rings of plates come to intervene between these primary radials (4) and the dorsocentral (1), as shown in Pl. I, fig. 12. They represent the basals, and occasionally also the under-basals, of a Crinoid, as I have already pointed out; and the former sometimes retain a considerable relative size in the adult form (Pl. I, figs. 3, 4, 5, 7; 3). In some species, too, the large "primary" plates which

<sup>1</sup> 'Trans. Roy. Soc.,' Edinb., vol. xxxii, p.

<sup>2</sup> Note V. This Journ., vol. xxii, p. 380.

<sup>3</sup> "Ophiuridæ and Astrophytidæ," 'Mem. Mus. Comp. Zoöl,' vol. i, No. 3.

<sup>4</sup> "Ophiuridæ and Astrophytidæ, New and Old," 'Bull. Mus. Comp. Zoöl,' vol. iii, No. 10, p. 264.

<sup>5</sup> 'Report on the "Challenger" Ophiuroidea,' p. 157, Pl. xl, figs. 11, 12.

surround the dorsocentral and form the "rosette" are not the radials (4) as they are in *Ophiomusium pulchellum* (Pl. I, fig. 9), or even in *Ophioglypha minuta* (Pl. I, fig. 8); but they are placed interradially as in *Ophioglypha lapidaria* (Pl. I, fig. 4), and correspond to the basals (3) of a Crinoid. In describing the disc of *O. minuta*, Lyman mentions "a central group of five primary plates whereof the middle one is pentagonal, while the five surrounding it are transverse oval;" and in *O. lapidaria* he speaks of "a close rosette, consisting of a pentagonal primary plate, surrounded by five others, smaller and of an irregular shape." He thus takes no account in his specific descriptions of the relative positions of the "primary plates," a point which might perhaps be of use when other characters were obscure. The manner in which these plates vary both in position and in development within the limits of a single genus is very remarkable, and I propose to dwell on it in some detail on account of its morphological bearings.

TABLE SHOWING THE VARIATIONS IN THE ARRANGEMENT OF THE APICAL PLATES IN THREE GENERA OF OPHIURIDS.

| Genus.                 | Radials large and in lateral contact.                              | Radials partly separated by Basals. | Radials completely separated by Basals.                          | Under-basals, Basals, and Radials. | Central Plates of Disc small, and without any definite arrangement. |
|------------------------|--------------------------------------------------------------------|-------------------------------------|------------------------------------------------------------------|------------------------------------|---------------------------------------------------------------------|
| <i>Ophioglypha</i> . { | convexa.<br>forbesi.<br>sculpta.<br>scutata.<br>solida.<br>undata. | <i>minuta</i> .                     | fasciculata.<br>imbecillis.<br>lacazei.<br>lapidaria.<br>tenera. | <i>abyssorum</i> .                 | costata.<br>lepida.<br>lovéni.                                      |
| <i>Ophiomusium</i> . { | lunare.<br>lütkeni.<br>pulchellum.<br>simplex.                     |                                     | flabellum.<br>granosum.                                          | scalare.<br>validum.               | cancellatum<br>laqueatum.                                           |
| <i>Ophiozona</i> . {   |                                                                    | marmorea.<br>tessellata.            | antillarum.<br>depressa.<br>insularia.                           | clypeata.                          | impressa.                                                           |

Let us begin by studying the principal structural variations that are to be met with in the arrangement of the apical plates on the disc of Ophiurids. The apical system of the following species remains permanently in an early embryonic condition. There are five radial primaries in close contact with one another, and with the dorsocentral just as in the young *Amphiura* or *Hemipholis* already mentioned.

|                                    |                                        |
|------------------------------------|----------------------------------------|
| <i>Hemipholis wallichi</i> , Dunc. | <i>Ophiomusium lunare</i> .            |
| <i>Ophioceramias clausa</i> .      | „ <i>lütkeni</i> .                     |
| <i>Ophioglypha convexa</i> .       | „ <i>pulchellum</i> (Pl.               |
| „ <i>forbesi</i> .                 | „ I, fig. 9).                          |
| „ <i>sculpta</i> .                 | „ <i>simplex</i> .                     |
| „ <i>scutata</i> .                 | <i>Ophiopholis aculeata</i> .          |
| „ <i>solida</i> .                  | <i>Ophiopyrgus saccharatus</i> , Stud. |
| „ <i>undata</i> .                  | „ <i>wyville-thomsoni</i> .            |
| <i>Ophiomastus secundus</i> .      | <i>Ophiotrochus panniculus</i> .       |
| „ <i>tegulitius</i> .              | <i>Ophiura carnea</i> .                |
|                                    | „ <i>stuwitzii</i> .                   |

N.B.—In this and subsequent lists the names given in the ‘“Challenger” Ophiuroidea’ have been used, except where an author’s name is appended.

The radials of the young *Amphiura* do not long remain in contact with the dorsocentral. For by the time that two adambulacral plates have appeared between them and the terminals they are separated from the dorsocentral by the rudimentary basal plates, while under-basals are developed very shortly afterwards. (Pl. I, fig. 12; 2, 3). In *Hemipholis cordifera*, however, and also in *Ophiura sarsi*,<sup>1</sup> more than a dozen arm-joints are formed before the radials lose their “primary” character and become hardly distinguishable among the small plates which cover the disc. Those of *Ophioglypha minuta* (Pl. I, fig. 8; 4) and *Ophiozona marmorea*,<sup>2</sup> although still large and prominent, are separated at their central ends by minute interradiial plates, the basals (3). In the follow-

<sup>1</sup> “Additamenta ad historiam Ophiuridarum,” i. Vid. Selsk. Skrifter, 5te Raekke. ‘Naturv. og. math. Afdeling,’ 5te Bind., Tab. i, fig. 3 a.

<sup>2</sup> “Report on the Ophiuroidea dredged by the ‘Blake,’” ‘Bull. Mus. Comp. Zool.’ vol. x, No. 6, Pl. iii, fig. 11.



ing species the basals are still larger and the proximal interbrachial plates are in immediate contact with them, so that the radial primaries do not meet one another at all.

*Ophiactis cuspidata*.  
*Ophioglypha fasciculata*.  
 „ *imbecillis*.  
 „ *lacazei*.  
 „ *tenera*.  
*Ophiolepis januarii*.  
*Ophiomastus texturatus*.

*Ophiopholis japonica*.  
*Ophiozona antillarum* (Pl. I,  
 fig. 6).  
 „ *depressa*.  
 „ *insularia*.  
*Ophiura albida*.  
 „ *squamosa*.

This separation of the radial primaries also occurs in *Ophioglypha lapidaria*, *Ophiomusium granosum*, and *O. flabellum*. (Pl. I, figs. 3, 4, 7). In all these three species the basals (3) and the radials (4) are about equal to one another, while in the last named they are also of nearly the same size as the dorsocentral (1). The “primary” plates immediately surrounding the dorsocentral are therefore interradial, and not opposite the arm-bases as “primaries” usually are. This difference is left unnoticed by Lyman, who speaks merely of a “central primary plate surrounded by five others” in *O. lapidaria*, and does not mention the real radial primaries (4) at all. The apical plates of the other two species are described as follows:—

*Ophiomusium granosum* (Pl. I, fig. 7). “In centre of upper surface of disc is a large pentagonal primary plate, surrounded by five others, quadrangular, and connected with the radial shields by five rudely triangular pieces; in each interbrachial space above are two quadrangular plates besides that of the margin.”

*O. flabellum* (Pl. I, fig. 3). “The central primary is pentagonal, surrounded by a row of angular plates, of nearly equal size; outside this row, in each interbrachial space, is another angular plate separating the inner ends of the rather long, closely-joined radial shields.”

In both these descriptions the basals (3) or angular interradial plates immediately next the dorsocentral (1) are referred to as “primaries;” while the true radial primaries (4), homolo-

gous with those of the young *Amphiura* (Pl. I, figs. 12, 13), or *Hemipholis*, and also with those of the adult *Ophiomusium pulchellum* (Pl. I, fig. 9), are not mentioned as such.

A yet more curious condition than that of these three species is presented by *Ophiomitra exigua* (Pl. I, fig. 5), which has no radials at all, nothing but the five interrarial basals (3) intervening between the dorsocentral (1) and the radial shields (*r*). Lyman<sup>1</sup> describes the disc as follows:—"Almost the entire upper surface is occupied by the large triangular, swollen, naked radial shields, which are joined their entire length. In the centre is a small patch of irregular scales with five radiating, single, interbrachial rows, and others along the margin."

The condition of this type is a very singular one, the more so as in other species of the genus no definite arrangement of the disc plates can be determined. Basals are present (Pl. I, fig. 5; 3) and radials absent; this being exactly the reverse of the ordinary embryological condition, which persists through life in so many species (Pl. I, fig. 9). It would be very interesting to know whether the radial primaries are ever developed and ultimately become resorbed, or whether they really never make their appearance at all. The "Blake" obtained only one specimen in 1877-78, which measures no more than 2.5 mm. across the disc; but this seems to be almost too large for the supposition that its radials have been greatly retarded in development, and that they would have appeared ere maturity was reached. Two of the "Blake" dredgings in 1879 yielded a type to which the same specific name is appended by Lyman, but with a (?), and he gives no information about them. The question, though unimportant as regards the Ophiurids alone, is one of some interest in its morphological bearings; for this type of "rosette" with interrarial primaries presents an approach to the condition of the calyx in an early Crinoid larva. This may have as many as eight stem-joints separating the dorsocentral from the relatively large basals by

<sup>1</sup> "Report on the Ophiurans obtained by the 'Blake,'" 1877-78, 'Bull. Mus. Comp. Zool.,' vol. v, No. 9, p. 231, Pl. i, fig. 5.

which the lower part of the cup is exclusively formed, the radials not having yet appeared in the equatorial zone between them and the orals. In the Asterids, too, the radials (Pl. I, fig. 15; 4) appear to develop late, long after the basals and the terminals. There can, I think, be little doubt but that these interradial plates, which are so often present in the "rosette" of Ophiurids, are identical with the five "intermediate" plates occupying a similar position in the young *Amphiura squamata* (Pl. I, fig. 12; 3) as figured by Ludwig. I have already pointed out<sup>1</sup> that I regard the latter as homologous with the basals of Crinoids which are marked 3 in all the diagrams given by Sladen and myself; and I have therefore designated the proximal interradial plates in the Ophiurid disc in the same manner, believing them to belong to the primitive abactinal system of the Echinoderm. According to Agassiz<sup>2</sup> the genital plates of the adult Ophiurid are formed from the angles of the primitive interradial plates. But the plates which he included in the latter category lay outside the circle of radial primaries, and not within the circle, between these primaries and the dorsocentral.<sup>3</sup> They are not comparable, therefore, to the proximal interradials in the abactinal system of other Echinoderms. These are called basals in Crinoids, and "genitals" in both Urchins and Starfishes. The latter name is an unfortunate one, as the genital ducts may have no relation to these plates even in an Urchin; and it is more than doubtful if any such relation exists in any Starfish. Agassiz<sup>4</sup> expresses himself very cautiously as to the identity of the primary interradials of the larva with the "ovarian plates" of a one-year-old Starfish. Ludwig tells us nothing on the subject, but Lovén<sup>5</sup> gives some excellent figures representing the position of these abactinal interradials in well-developed individuals of four species of Asterids, three of

<sup>1</sup> This Journal, vol. xxii, 1882, pp. 378—380.

<sup>2</sup> 'North American Starfishes,' p. 93.

<sup>3</sup> See this Journal, vol. xxii, 1882, pp. 380, 381. Note.

<sup>4</sup> 'North American Starfishes,' pp. 51, 52.

<sup>5</sup> 'Études sur les Échinoidées,' pp. 88, 89.

which also show the under basals very clearly. Speaking of them as "costals,"<sup>1</sup> he says, "Il est vrai que les conduits efférents des organes de la génération, chez les Echinoidées en possession, toutefois incertaine, des costales, en sont relégués définitivement chez les Astériadées et rejetés dans le périsome interrâdial." Ludwig continually speaks of the "genital plates of Urchins and Asterids," though he evidently intends to assert nothing but their homology in the two groups. But in one passage,<sup>2</sup> at any rate, he distinctly implies that these plates are sometimes related to the genital openings in Starfishes just as in the regular Urchins. The genital plates or scales of Ophiurids are of another nature altogether, and may correspond, as stated by Agassiz, to the interbranchial partitions of Starfishes;<sup>3</sup> but they must not be confounded with the so-called "genitals" in this group and in the Urchins. As regards the Asterids, the name appears to be altogether undeserved; while Lovén's observations, supported by those of Sladen, seem to indicate clearly that the plates in question remain in the neighbourhood of the dorsocentral, like their homologues in the Ophiurids and Urchins, though occasionally further removed from it. The following passage from Agassiz is interesting in this connection. "But we have, in a great many genera of Starfishes, the central part of the disk, showing, in the young stages only, as regular an arrangement of the plates of the abactinal system as in any Ophiuran, though it is lost in the adult." Sladen's observations show, however, that this regular arrangement may persist for a considerable time; and he has wisely discarded the use of the word "genitals" for the primary interrâdial plates of the abactinal system of a Starfish.

Let us now pass on to a consideration of those types in which the under-basals, already noticed in the young *Amphiura* (Pl. I, fig. 12; 2), are traceable in the apical system

<sup>1</sup> For some remarks on the use of this name, see this Journal, vol. xviii, 1878, pp. 363—365.

<sup>2</sup> 'Morphol. Stud.,' ii, p. 79.

<sup>3</sup> 'North American Starfishes,' pp. 93, 103.



of the adult. It will be remembered that Ludwig places them in the category of intermediate plates. I think that they may be identified with tolerable certainty in the following species, while there are others in which their presence is less easy to determine.

|                             |                       |
|-----------------------------|-----------------------|
| Ophioceramis clausa (Pl. I, | Ophiomusium scalare.  |
| fig. 1).                    | „ validum (Pl. I,     |
| Ophioglypha abyssorum.      | fig. 2).              |
| Ophiozona clypeata.         | Ophiolepis variegata. |

The last species is perhaps out of place in this list. The dorsocentral is surrounded by three alternating rows of plates, all of them about the same size; and it is a little difficult to decide whether the proximal row should be regarded as under-basals or as the radial primaries. In the latter case the plates of the second row, instead of being basals, would belong to the interbrachial system; and they may fairly be compared to the interradians of the Palæocrinoidea and of the recent *Thaumatoocrinus*.

It will be remembered that in the early stages of the development of a Crinoid two of the first radials are separated from one another by an anal plate, which rests directly on one of the basals. It subsequently becomes lifted out of the calyx and is eventually totally resorbed. But its earlier condition is permanent in many Palæocrinoids such as *Hexacrinus* and *Dichocrinus*; and a possible parallel to it may be found in the *Ophiura stuwitzi* of Lütken,<sup>1</sup> which has six "primary" plates in contact with one another around the dorso-central. Five of these are opposite the arm-bases, and are therefore the radial primaries; while the sixth is interradian and occupies exactly the same relative position as the anal plate of *Dichocrinus* and *Actinocrinus*.

In the last-named genus, and in a large number of other Palæocrinoids such as *Platycrinus*, *Glyptocrinus*, and their allies, there are primary interradians all round the calyx, resting upon the upper faces of every two contiguous radials, but

<sup>1</sup> 'Add. ad. hist. Ophiur.,' loc. cit. i, Tab. i, fig. 8 a.

not separating them laterally as the anal plate does. These plates reappear in a few Neocrinoids such as *Guettardicrinus* and *Apiocrinus roissyanus*, where they form part of the calyx just as in the Palæocrinoids; and their representatives are readily distinguishable in *Ophioglypha minuta*, and *Ophiomusium pulchellum* (Pl. I, figs. 8, 9; 1), as in all those Ophiurids in which the large radial primaries meet one another laterally and form a complete ring around the dorsocentral.

But in the recent *Thaumatocrinus*,<sup>1</sup> as in the Palæozoic *Rhodocrinus*, *Thylacocrinus*, and other genera included by Wachsmuth and Springer in the section *Rhodocrinidae*, the primary interradial plates rest directly upon the basals and completely separate the first radials from one another all round the calyx; and this indicates the persistence of an exceedingly early developmental condition. A precisely similar arrangement is presented by those Ophiurids in which the radial primaries do not meet one another laterally, but are separated by the proximal interbrachial plates, e.g. *Ophioglypha lapidaria* (Pl. I, fig. 4), *Ophiomusium flabellum* (fig. 3), *O. granosum* (fig. 7), and *Ophiozona antillarum* (fig. 6). These proximal interbrachials (1) rest directly upon the basals (3), which are situated inside the circle of primaries (4).

The radials of the Neocrinoid are primitively separate (Pl. I, fig. 11; 4) but ultimately approach one another and form a closed ring (fig. 10); whereas in the Ophiurid they are at first in close lateral contact both with one another and with the dorsocentral (fig. 13). In some species (figs. 1—4, 6—8) they subsequently become separated by the intercalation of Ludwig's intermediary plates, *i. e.* the basals, under basals, and the proximal interbrachials, as shown by Ludwig's figures of *Amphiura* (Pl. I, fig. 12). This separation of the radial primaries in Ophiurids is readily explained by their want of any relation to important internal organs, such as is characteristic of the radials of a Crinoid, which protect more or less

<sup>1</sup> "On a new Crinoid from the Southern Sea," 'Proc. Roy. Soc.,' No. 225, 1883.

of the chambered organ and the lower part of the glandular structure arising from it. In fact it is the absence of relationship to internal organs which markedly distinguishes the plates in the apical system of Ophiurids and Asterids from their homologues in Urchins and Crinoids. In the former group the basals are frequently pierced by the genital ducts and the radials (oculars) pierced by the water vessels. In the Crinoids, however, the chambered organ (which is so important a part both of the vascular and of the nervous systems) is lodged in a cavity bounded by the basals and radials; while the lower part of the plexiform gland arising out of it passes upwards within the radial funnel. The primary cords proceeding from the chambered organ pierce the basals; and the secondary cords resulting from their bifurcation enter the radials, where they are connected by the circular commissure, and then pass on to the arms.

Some palæontologists have asserted that the basal plates are absent in the Eugeniocrinidae and Holopodidae, thereby suggesting very considerable morphological difficulties, as I have pointed out elsewhere;<sup>1</sup> and I have yet to become acquainted with a Crinoid in which the existence of basals, though perhaps not actually demonstrable, cannot be deduced from the analogies presented by the calyx to that of *Rhizocrinus lofotensis*, *Bathycrinus carpenteri*, and other forms in which the interbasal sutures disappear when maturity is reached.

A parallel condition to the variations in development and functional unimportance of the plates in the apical system of the Stellerids is presented by the oral system of certain Palæocrinoids, e.g. the Platycrinidae, Actinocrinidae, and Rhodocrinidae. In these families a regular "calyx" is developed around the left larval antimer, like that which appears on the right antimer of all *Pelmatozoa* and *Echinozoa*. In the Neocrinoids, as in the Ophiurids and in the Psolidæ among *Holothurians*, five "oral" plates are developed around

<sup>1</sup> "On the Supposed Absence of Basals in the Eugeniocrinidæ, and in certain other Neocrinoidea," 'Ann. and Mag. Nat. Hist.,' May, 1883, pp. 327—334.

the left vaso-peritoneal tube of the larva. These are inter-radial in position and correspond to the basals, which are developed in the same manner around the right vaso-peritoneal tube in Urchins, Stellerids, and Crinoids. The oral plates of the Crinoid larva primitively close in the peristome, but eventually separate so as to expose the mouth and tentacles to the exterior. But in the Palæocrinoids the mouth remained subtegmenal; while a central plate corresponding to the dorso-central appeared within the circle of orals (apical dome plates of Wachsmuth). In some genera, e.g. *Symbathocrinus* and *Haplocrinus*, the oral or actinal system remained permanently in this condition;<sup>1</sup> but in more complex types radial and interradial dome plates were developed outside the orals, in many cases corresponding plate for plate with those forming the calyx below. There is, however, even within generic limits, much less regularity in the size and grouping of the plates forming this oral or actinal system than there is in the abactinal calyx plates. This will be readily understood if we remember that the former were not traversed, as the basals and radials were, by motor nerves proceeding outwards from a central organ. Sometimes, indeed, as in *Strotocrinus*, the oral plates and primary radial dome plates become indistinguishable from the numerous other minute plates which form the vault. This condition finds a close parallel in many Ophiurids in which the whole surface of the disc is crowded with minute scales, and the rosette of "primary" plates cannot be made out; though they are visible enough in young individuals (Pl. I, figs. 12, 13) just as the proximal dome plates (orals) are in the vault of the young *Strotocrinus* or *Megistocrinus*.

The extremely Crinoidal appearance of the apical plates of

<sup>1</sup> I am greatly indebted to Mr. Wachsmuth both for new information respecting the structure of the summit in these two genera, and also for the opportunity of examining many excellent specimens illustrating this and other points. But his interpretation of the plates is somewhat different from that here given, which is based on general embryological considerations. These will be found detailed at length in this Journal, vol. xix, pp. 180—184.



*Ophiomusium granosum* (Pl. I, fig. 7) will be universally admitted; and so, though in another way, with the curious genus *Ophiopyrgus*. Lyman<sup>1</sup> says of it, "With its peg-like central primary plate and dome-like disk it suggests a simple-armed Crinoid whose head has been broken from the stem." Studer<sup>2</sup> speaks to the same effect; but he has been singularly unfortunate in his more detailed comparison between the apical plates of Ophiurids and Crinoids respectively. Adopting the views of Lovén and Agassiz, as modified by myself,<sup>3</sup> concerning the homology of the so-called "genital plates" of Urchins and Starfishes with the basals of a monocyclic Crinoid, he compares "die Anlage des Skeletts bei Ophiuriden" with the calyx of a dicyclic Crinoid. This is, in fact, what I did myself in my fifth note,<sup>4</sup> but our methods of procedure are radically different.

*Ophiopyrgus* is a genus which resembles *Ophiomusium pulchellum* (Pl. I, fig. 9) in having a dorsocentral (1) surrounded by five radial primaries (4), but no basals. According to Studer, "Bei *Ophiopyrgus* ist das Centrodorsale mit seiner erhabenen Form noch dem eines ungestielten Crinoiden, etwa *Haplocrinus*, ähnlich. Dann folgen die radialen Basalia, die Radialia sind zum Terminale geworden und an der Spitze der Arme gerückt, während die Parabasalia nach der Ventralseite gerückt sind und zu Mundschildern wurden. Auch hierzu bietet *Haplocrinus rosaceus*, Roem. Analogieen, indem dort die Parabasalia schon eine actinalgeneigte Stellung einnehmen und unmittelbar die grossen Oralplatten tragen."

The piece which Studer calls centro-dorsal is that termed dorsocentral by Sladen and myself, the former term being reserved for the top stem-joint of the *Comatulæ*, to which it was originally limited by Dr. Carpenter. This is a totally different

<sup>1</sup> "Challenger" Report, p. 33.

<sup>2</sup> 'Uebersicht über die Ophiuriden welche während der Reise S. M. S. Gazelle um die Erde 1874—1876 gesammelt wurden,' 'Abhandl. d. Königl. Preuss. Acad. d. Wiss. zu Berlin,' 1882, p. 10 (of separate copy).

<sup>3</sup> This Journal, vol. xviii, 1878, pp. 356—371.

<sup>4</sup> This Journal, vol. xxii, 1882, pp. 378—381.

structure, as I have explained elsewhere.<sup>1</sup> Studer refers to the figure of *Haplocrinus rosaceus*, Roem. on p. 347 of Zittel's well-known work on Palæontology. Two species are figured on this page, viz. *Coccocrinus rosaceus*, F. Roem., and *Haplocrinus mespiliformis*, Goldf., both from the Devonian beds of the Eifel. Neither of them is "ungestielt," as may be seen by reference to Taf. xii, in Schultze's well-known 'Monographie der Echinodermen des Eifler Kalkes,' from which Zittel copied his figures; while on pp. 89 and 105, Schultze describes the stem-characters of the two species. Studer's subsequent remarks indicate clearly that *Haplocrinus* should read *Coccocrinus*, and that he is comparing the disc of *Ophiopyrgus* with the calyx of *Coccocrinus rosaceus*. Unfortunately, however, this species does not possess a dicyclic base. For the plates which Studer calls Parabasals, describing them as having assumed an actinal position and supporting the orals are the primary interradians. These rest on the upper angles of every two contiguous radials; whereas if they were "parabasals" they should be situated between radially placed "basals" (which are not present) and the radials themselves; and one of the radials would rest on the upper angles of every two of these plates, this being exactly the reverse of their real position!

Studer's comparisons further imply that the terminal plates of the Ophiurid arm are after all the homologues of the Crinoid radials; but this is the very point which Ludwig disproves in the useful memoir which Studer quotes. The plates which Ludwig recognised as the radial primaries (Pl. I, fig. 13, 4), are considered by Studer to be the lower ring of basals (under-basals of the rational nomenclature); this being necessary to establish an homology between an Ophiurid and a dicyclic Crinoid. My own attempt at an homology between Ophiurids and Crinoids is of later date than the presentation of Studer's memoir to the Berlin Academy, but anticipated its publication by nearly six months. As he has added no note upon the subject, I conclude that he did not know of my

<sup>1</sup> This Journal, vol. xviii, 1878, pp. 372, 373.

paper,<sup>1</sup> which is unfortunate; for I think it might have led him to reconsider his statements. It is there pointed out that the Ophiurid may be compared to a dicyclic Crinoid without the necessity of regarding the radial primaries as under-basals, and the mouth-shields as parabasals, or basals proper. Studer does not appear to adopt the views of Ludwig and myself respecting the homology of these mouth-shields with the orals of Crinoids. For though he calls them "parabasals" he also gives this name to the primary interradiial plates which support the orals of *Coccoerinus*. As I have already pointed out, these are above the radials instead of below them, as they should be if they are to represent the parabasals of true dicyclic Crinoids such as *Poteriocrinus*. They are not called parabasals by Zittel, whom Studer quotes, nor by Schultze; and I cannot make out that any other palæontologist has ever spoken of them by this name. In every Crinoid with a dicyclic base the upper row of plates rests directly upon the lower. Whereas in *Coccoerinus*, the type selected by Studer, the so called parabasals are separated from the basals by the ring of primary radials. It is only by employing this novel terminology that Studer is able to carry out his comparison between the Crinoid and Ophiurid; while the position which he assumes, intermediate between those of Ludwig and myself, is a very singular one. He adopts Ludwig's view respecting the homology between the mouth-shields of Ophiurids and the genitals of an Urchin, on account of the "dorsal origin" of the former and their relation to the water-vascular and blood-vascular systems; but he agrees with me in comparing the parabasals of dicyclic Crinoids<sup>2</sup> and the basals of *Pentacrinus* to these same genital plates in the Urchin, and the first formed interradials in the young Starfish. Consequently the parabasals of the dicyclic Crinoids represent the mouth-shields of Ophiurids. It is this conclusion which he reaches (verbally) in his reasoning on *Coccoerinus*, where,

<sup>1</sup> This Journal, vol. xxii, October, 1882, pp. 378—381.

<sup>2</sup> I presume that Studer is here referring to true dicyclic Crinoids like *Poteriocrinus* and *Marsupites*, and not to *Coccoerinus*.

however, he is using the word parabasals in a new sense. But it is obviously the one which he intended; for he speaks of the parabasals (basals mihi) as passing over to the ventral side and becoming mouth-shields. Apparently, therefore, he does not find any homologues in the Ophiurids for the orals of Crinoids; and yet the relation of these plates to the vascular systems is the same as that of the mouth-shields in Ophiurids as was pointed out by Ludwig. His comparison of the dorso-central of *Ophiopyrgus* with that of a stemless Crinoid shows that he considers the dorsal regions homologous in Crinoids and Ophiurids; but at the same time he compares plates which are ventral in the Ophiurids with others which are dorsal in the Crinoids. This seems to be on account of the so called "dorsal origin" of the mouth-shields, a point which I believe to be more apparent than real. They can only be called dorsal if they are developed around the right vasoperitoneal tube, which is as yet not proved; and I more than doubt if it ever will be.

Studer follows Zittel in retaining the name "parabasals" for the upper row of plates beneath the radials of dicyclic Crinoids. The latter author<sup>1</sup> fully accepts the homology between them and the "basals" of the monocyclic forms; but he thinks that the change of terminology necessitated by calling them basals would lead to confusion, owing to this name having been previously applied to the lower ring of plates on which the parabasals rest. There is, indeed, a certain advantage in the retention of the name parabasals, as it indicates at once that the type under consideration is one with a dicyclic base. But this indication is already given in the expression under-basals; and the use of two names for one and the same set of plates seems to me calculated to mislead future workers, who will not be, as we are now, in a transitional period between empirical and rational methods of nomenclature. The bones of the carpus and tarsus, or those of the skull are not renamed for every variation in number and arrangement which they present. Certain elements are constant and others variable.

<sup>1</sup> 'Handbuch der Palaeontologie,' i Band, pp. 327, 328.



The former always receive the same names, and the latter are baptised according to circumstances. Why then should not the plates which alternate with and support the radials of a Crinoid always receive the same name, whether they rest directly on the stem or not? The following table shows the variety of designations which have been applied to the two sets of plates beneath the radials of dicyclic Crinoids since the time of Müller.

|                                  | Monocyclic Crinoids. | Dicyclic Crinoids. |                      |                     |                   |                       |
|----------------------------------|----------------------|--------------------|----------------------|---------------------|-------------------|-----------------------|
|                                  |                      | Müller.<br>1843.   | De Koninck.<br>1854. | Carpenter.<br>1878. | Zittel.<br>1880.  | Trautschold.<br>1882. |
| Proximal Ring. {<br>Radial. }    | Absent.              | Basals.            | Basals.              | Under-<br>basals.   | Infra-<br>basals. | Infra-<br>basals.     |
| Distal Ring. {<br>Interradial. } | Basals.              | Para-<br>basals.   | Sub-<br>radials.     | Basals.             | Para-<br>basals.  | Supra-<br>basals.     |

As if this question of nomenclature were not sufficiently complicated already, a further change has been proposed by Trautschold,<sup>1</sup> who wishes to call the subradial plates of dicyclic Crinoids "supra-basals." His reasons are that the "Basis" of the monocyclic forms consists of but one ring of plates; for while "bei den dicyclischen die zwei unteren Tafelkränze die Basis bilden, so sind die Platten beider gleichwerthig, und die Platten der ersten wie der zweiten Basis sind basalia oder Basalplatten, sie sind integrirende Theile der monocyclischen Basis sowohl wie der dicyclischen."

The question here turns on the use of the term "Basis," which Trautschold interprets as meaning all plates between the radials and the top stem-joint; and in this sense there is an undeniable equivalency between the single and double sets of subradial plates in the two forms of calyx. But the dicyclic

<sup>1</sup> "Ueber die Bezeichnung der Kelchplatten der Crinoideen," 'Bull. Soc. imp. des Nat. de Moscou,' Tom. lvii, 1882, No. 3 (1883), pp. 201—203.

base as a whole is not homologous with the monocyclic base; for it contains radially situated plates which are absent in the latter. The variations of the apical system in Ophiurids and Asterids throw much light upon this question; for while some forms have one ring of plates within the radial primaries (Pl. I, figs. 3, 4, 6—8, 15), others have two (figs. 1, 2, 12, 14, 16), and others none (figs. 9, 13). But it would be wholly incorrect to say that the plates marked 2 and 3 in *Ophiomusium validum* (fig. 2) were collectively homologous to the single plates marked 3 in *O. flabellum* or *O. granosum* (figs. 3, 7). The logical result of this would be that both these sets of plates, together with their respective radial primaries (4), are collectively homologous with the radial primaries of *Ophiomusium pulchellum* (fig. 9; 4) or the interradiial primaries of *Ophiomitra exigua* (fig. 5; 3); for the latter are the only plates intervening between the radial shields and the dorsocentral.

It appears to me that the question turns upon the often forgotten difference between homology and analogy. Trautschold remarks: "Basalplatten zu nennen, die sich in der Mitte zwischen dem unteren und oberen Plattenkreise befinden, wird immer paradox erscheinen, ganz unerklärlich aber dem, der noch nicht in die Feinheiten des Baues der Echinodermen und in die subtilen Deductionen der Forscher eingeweiht ist." This is doubtless true, but is it not more confusing to multiply names unnecessarily? The term "under-basals" explains itself and is at once indicative of a dicyclic type.

All writers have agreed that some alteration of the Müllerian terminology is necessary to the scientific study of Crinoids. Probably the least paradoxical change would have been to retain De Koninck's name, subradials, for the parabasals of dicyclic forms, and also to transfer it to the basals of the monocyclic types. The name "basals" would thus be retained only for those plates which occur in dicyclic Crinoids and are now generally called "under-basals." I considered this question carefully before proposing to use the terms basals and under-basals for the plates of the dicyclic base; but was met

by the following difficulty. Müller<sup>1</sup> originally proposed the name *basals* for those plates beneath the radials of *Pentacrinus* "die mit den Kelchradien alterniren." They actually do form the base or lowest part of the calyx in the great majority of Crinoids; and it therefore seemed inexpedient to retain this name only for plates which are radial in position and of less frequent occurrence, although it had been erroneously applied to them by Müller. This would certainly have been a greater change than the one which I proposed, though probably less paradoxical to non-morphologists. But it would have inevitably have led to the belief that a "Basis" is present in comparatively few Crinoids, instead of being universal as Müller described it.

There can be no true "Science of Ancient life" which is not based upon the facts and deductions of morphology, subtle as the latter may appear to be to the collector. For without them palæontology degenerates into the compilation of empirical descriptions of fossils, accompanied by the details of their geological positions; and these, however important to the geologist, are almost entirely devoid of any value to the biologist. How could Cope, Marsh, Leidy, and other well-known American palæontologists, properly describe the marvellous new generic types which they so often discover, without a rational system of nomenclature based upon vertebrate morphology? Considerations such as these led me to the use of the terms *basals* and *under-basals* for the two rings of plates between the stem and the radials of *diyclic* Crinoids; and they have been adopted, I am glad to say, by Wachsmuth and Springer, Barris, Wetherby, H. S. Williams, de Loriol, and other writers who are frequently describing new Crinoids.

One point more is worth noticing. Lovén, Wachsmuth, and Springer, and Agassiz consider the *under-basals* as representing the dorsocentral plate of the young Urchin. I have already endeavoured to show that this position is untenable, and would urge one more argument against it. Not only has *Marsupites*

<sup>1</sup> "Ueber den Bau des *Pentacrinus caput-Medusae*," 'Abhandl. d. Berlin Akad.,' p. 25 (of separate copy).

a dorsocentral plate as well as under-basals, but the same is the case with some Ophiurids, as I have pointed out above (Pl. I, figs. 1, 2, 12), and with many Asterids as shown by Sladen (Pl. I, figs. 14, 16). Wachsmuth and Springer<sup>1</sup> seem to have felt this difficulty; for they do not, as Lovén and Agassiz did, regard the plate within the ring of under-basals as a dorsocentral; but they are inclined to think that it represents the column of Crinoids generally. This supposition, however, is not necessary when the condition of the apical system of Stellerids is taken into consideration, as is shown both in the preceding and in the following pages.

POSTSCRIPT.—Since the above was written, I have received, by the kindness of Prof. Agassiz, a copy of the beautiful Atlas of plates illustrative of the embryology of Echinoderms.<sup>2</sup> Fortunate, indeed, is the student who has access to this most useful work. But, without in any way desiring to be hypercritical, I could wish that a little more uniformity and precision of nomenclature had been adopted for the plates of the Apical System.

The cirrus-bearing top stem-joint of *Comatula* receives its proper name, *centrodorsal*, as used by Sir Wyville Thomson and Dr. Carpenter. But this name is also applied to the central plate in the Apical System of the Stellerids, which is another structure altogether, as I have often stated in the pages of this Journal. Ludwig invariably spoke of it as the “Centrale,” while Lovén called it the “Dorsocentral,” as do Sladen and myself. In some copies of Ludwig’s figures, however, which appear in the Atlas, it is marked “Dorsocentral,” and in others “Centrodorsal.” But since the Echinozoa are stemless, and, consequently, have no Centrodorsal, I fear that this uncertainty of nomenclature cannot but lead to confusion in the mind of the learner who has been previously studying the earlier plates of Crinoid embryology.

For the same reason I regret the insertion of the word

<sup>1</sup> “Revision of the Palaeocrinoidea,” part i, p. 22, ‘Proc. Acad. Nat. Sci.’ Philad., 1879.

‘Mem. Mus. Comp. Zool.’ vol. ix, No. 2.



“basal,” in brackets, after “Dorsocentral,”<sup>1</sup> in the explanatory description of a copy of Lovén’s figure of a young *Asterias glacialis*. I have pointed out more than once, and Sladen has expressed the same views, that it is impossible to arrive at a rational morphology of the Echinoderm apical system, if a primitively single plate “Centrale” is to be considered as homologous with five peripheral ones, whether they be radial or interradial, under-basals or basals.

<sup>1</sup> “Atlas,” p. 27.

## On the Homologies of the Primary Larval Plates in the Test of Brachiote Echinoderms.

By

**W. Percy Sladen, F.L.S., F.G.S.**

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With Plate I.

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ALL that had previously been written upon the subject of homologous plates in larval Echinodermata was critically discussed in the pages of this journal by my friend Mr. P. Herbert Carpenter, in his able and philosophical memoir "On the Oral and Apical Systems of Echinoderms,"<sup>1</sup> and in his succeeding papers on Echinoderm Morphology.<sup>2</sup> It will therefore be unnecessary for me to refer historically to the conflicting views which have been held at different times by those naturalists who have devoted their attention to the comparative embryology of the group, and who have endeavoured to trace correspondent plates in the different orders. In addition to systematising and consolidating what was known, Carpenter has also extended our knowledge of the subject greatly, not only by means of direct research but also by furnishing an interpretation of observations made by others, from which logical deductions had not previously been drawn. As my friend's papers were written essentially from the Crinoid standpoint, I propose in the present notes to approach the subject from the Asterid side, because in the first place I

<sup>1</sup> Vol. xviii, 1878, p. 351, and vol. xix, 1879, p. 176.

<sup>2</sup> Vol. xx, 1880, p. 321; vol. xxi, 1881, p. 169; vol. xxii, 1882, p. 371.

differ from the conclusions arrived at by some of my predecessors, and in the second place I trust to be able to bring forward some details in the structure of the Asteroidea which, in my opinion, elucidate difficulties that have hitherto been the theme of doubtful contention and diversity of opinion.

In studying the homologies of the primary calcareous elements of the test, much difficulty and some inaccuracy has hitherto arisen from ignoring the fact that the corresponding plates in the different orders of Echinoderms do not appear at the same period of growth in the larva of each, or, in other words, that the first appearance of a given series of primary test plates occurs at a different stage of the development of the larva in relation to the other series of plates in the different orders; e.g. in Ophiurids the radial plates are generally well developed before any trace of the basals appear, whilst in the Asterids the basals have already attained a large size before the primary radials are represented. To this subject I shall have occasion to refer again presently.

In the diagrams given on Plate I the respective larvæ are represented at a stage when all the so-called primary plates are present and consequently when due comparisons can be made between them. I shall now proceed to remark briefly upon a type from each of the orders.

The Crinoid as the standard of comparison demands our first attention. Fig. 10 represents a diagram of the plates of the young *Antedon rosacea* at the stage just before separation from the stem takes place. The respective plates are lettered in accordance with the nomenclature proposed by Herbert Carpenter,<sup>1</sup> and with which I entirely agree; his deductions being perfectly logical and in my opinion conclusive. The first radials (4, 4) are enlarged and enclose the circle of smaller basal plates (3, 3,) excluding them entirely from touching the equatorial zone. The centro-dorsal plate has been removed and its position is indicated by the space marked 1, which also represents the telescoped stem and radical plate or root-disk,

<sup>1</sup> "Oral and Apical Systems of the Echinoderms," 'Quart. Journ. Micr. Sci.,' vol. xviii.

the latter being equivalent to the dorsocentral plate throughout the series. The brachial plates are in process of formation. The oral plates, which are entirely on the actinal hemisome, are separated from the abactinal hemisome by the development of the intermediate perisomatic membrane and are rapidly being resorbed.

At a much earlier stage of development than the one we have been considering, the circle of basal and the circle of oral plates are alone present, and constitute respectively the whole of the actinal and abactinal hemisomes of the calyx. At a later stage the radials are developed, and their first appearance is figured by Allman,<sup>1</sup> Thompson,<sup>2</sup> and Dr. Carpenter.<sup>3</sup> They are represented in Herbert Carpenter's diagram,<sup>4</sup> given here in fig. 11.

Comparing this stage with the one given in fig. 10, it is interesting to note the astonishing alterations which have taken place during the process of development in the relative proportions of the various plates; the chief feature being the enormous development of the first radial plates, which have increased in size at a much greater ratio than any of the other plates.

Passing next to the Ophiurid larva, as in many respects the nearest phylogenetic ally of the Crinoid, we are able to indicate with little hesitation the plates which are homologous to the radial plates of the Crinoid. They are situated on the disc between the base of the rays and the dorsocentral plate (marked 4 in fig. 12). Adcentral to the circle of radial plates is another circle of interradially placed plates, which are equivalent to the basals of a Crinoid (marked 3, 3, in the diagram). In the form under notice still another inner ring of plates is present; these are radially placed and are homologous with the under-basals of the dicyclic Crinoids (e.g.

<sup>1</sup> 'Trans. Roy. Soc. Edin.,' vol. xxiii.

<sup>2</sup> 'Phil. Trans.,' vol. clv, pl. xxv, et seq.

<sup>3</sup> 'Phil. Trans.,' vol. clvi, pl. xxix.

<sup>4</sup> 'Quart. Journ. Micr. Sci.,' vol. xxii, "Notes on Echinoderm Morphology," No. v, p. 11 (separate copy), fig. iii.



*Enerinus*, *Cyathocrinus*, &c.), but which are not represented in the *Comatula* larva. The under-basals are marked 2, 2, in the diagram. At the extremity of the rays are found the odd terminal plates *r*, *r*. These plates, in my opinion, have no representatives in the Crinoid.

Ludwig in his admirable memoir on the development of the Ophiurid skeleton,<sup>1</sup> states that he is unable to say positively whether the terminal or the first radial plate is the first formed; and it is unquestionable that there is very little difference in the time of the appearance of the two sets of plates in the species he studied, *Amphiura squamata*,—an example, it may be remarked, of a viviparous Ophiurid in which the development is abbreviated. As far as judgment can be drawn from the the figures given by Agassiz,<sup>2</sup> Metschnikoff,<sup>3</sup> and Müller<sup>4</sup> of larval Ophiurans which pass through a pluteus stage it seems more probable that traces of the terminal plates appear before the first radials are formed. Although in the case of *Amphiura* studied by Metschnikoff (op. cit., Taf. iv, fig. 17), and perhaps in some of Müller's figures also, the reverse would seem to occur. The relation of the first radials to the water-vascular system in Metschnikoff's figure is very noteworthy. The question, however, whether the terminals or the plates which we have called first radials in the Ophiurid are the first to appear, has, in my opinion, upon phylogenetic and embryological grounds, nothing whatever to do with the determination of the homologues of the Crinoid first radial, although some writers would seem to infer such a view by saying that in Asterids and Ophiurids the first radial (sic) is carried out during growth to the extremity of the ray.

The primitive structure and mode of formation of the terminal plate is different from that of the first radial.

<sup>1</sup> 'Zeitschr. f. wissenschaft. Zool.,' Bd. xxxvi; 'Morphol. Studien,' ii, p. 104.

<sup>2</sup> "On the Embryology of the Echinoderms," fig. 32, 'Mem. Amer. Acad.,' 1864.

<sup>3</sup> "Ueb. die Entwicklung der Echinodermen u. Nemertinen," Taf. vi, vii, 'Mem. Akad. St. Petersburg,' 1869.

<sup>4</sup> "Ueb. Ophiurenlarven des Adriat. Meeres," Taf. iii, iv, vii, 'Berlin Akad.,' 1851.

The plates marked 4, 4, in the diagram given in fig. 12, are called "primary radials" by Ludwig,<sup>1</sup> and he also remarks:—"Was nun die fünf primären Radialia des Scheibenrückens anbelangt, so sind dieselben nicht nur bei *Amphiura squamata*, sondern auch bei anderen Arten von früheren Forschern bereits öfters erwähnt worden." Carpenter<sup>2</sup> has clearly asserted their homology with the first radials of a Crinoid. In further support of this view it should be borne in mind that not only these radial plates, but also companion plates homologous with the other elements of the Crinoid calyx, are persistent throughout the adult stage of a very large number of Ophiurans. Upon this subject Carpenter has prosecuted some very interesting investigations, which are published in another page of this Journal (*ante*, pp. 4—11).

On the strength of these considerations it seems to me irrational to regard the terminal plates  $\tau, \tau$ , as the homologues of the first radials of the Crinoid calyx, for we should then be compelled to call the plates 4, 4, in fig. 12, second radials, and we should be placed in the anomalous position of having a ray in which the second radial was proximal in relation to the first radial; a state of things which in the adult would be further emphasised by the separation of those two plates by the whole series of brachial plates. I have alluded to this subject at somewhat greater length than might perhaps be thought necessary for reasons which will be apparent when we discuss the homologies of the plates in the next group.

In the Ophiuran at the stage given in fig. 12, the plates which ultimately become the mouth-shields are entirely on the actinal hemisome, but have not yet reached their permanent position. These plates are homologous with the oral plates of a Crinoid. One of the mouth-shields (orals) bears the water-pore.

Turning now to the earlier stages of the development of the Ophiurid under notice (*Amphiura squamata*), we find that the first plates formed on the abactinal hemisome are

<sup>1</sup> Op. cit., pp. 104, 105.

<sup>2</sup> "Notes, v," op. cit., pp. 9, 10, figs. i and ii.

the radials and the terminals. The orals and some of the accompanying plates of the actinal hemisphere next appear, before any trace of the dorsocentral plate is present. At the stage when the dorsocentral is finally formed, the plates above-mentioned constitute the whole of the plating of the test. It is interesting to compare the great preponderance of the radial plates at this early period of the young Ophiuran (given in diagram in fig. 13) with the proportions of the same plates in the Crinoid at a much later period of growth (fig. 10). It almost seems like an exaggeration of the character of the same plan of development and suggests the idea that the young Ophiurid commenced its development with an exaggerated presentment or ultimatum of what had been the general scheme and whole tenour of the process of development as exhibited in a late stage of the young Crinoid larva.

At a later stage the homologues of the basal plates appear, internal to, and alternating with, the circle of radials; and these are followed by other intermediate and interradially placed plates. Ultimately the under-basals are developed (fig. 12; 2).

Proceeding now to the consideration of the Asterid larva, little difficulty will be found in the stage we have selected for fig. 14 in homologating the various plates with those of the two preceding groups. Here as in the larval Crinoid and Ophiurid we have a dorsocentral plate (1), surrounded by a circle of five interradially placed plates (3, 3) of large size in the Asterid, corresponding to the basal plates of the Crinoid. Outside and alternating with these are five smaller plates (4, 4), radially placed, which I consider to be homologous with the first radials of the Crinoid calyx. At the extremity of the rays are large terminal plates ( $\tau$ ,  $\tau$ ). These plates are homologous with the terminal plates which occupy a similar position in the Ophiurid, and like them are, in my opinion, without a representative in the Crinoid.

All previous writers who have dealt with this subject have, without exception, considered the terminal or so-called ocular plate at the extremity of the ray of an Asterid as homologous

with the first radials of the Crinoid calyx. (The plate marked  $\tau$  in fig. 14, with the plate marked 4 in fig. 10.) This view I hold to be erroneous and base my conclusions upon the following grounds.

On comparing the Asterid diagram (fig. 14) with the Ophiurid diagram (fig. 12), or even the younger stage given in fig. 15 with that in fig. 13, no one, I imagine, would dispute the perfect homology that exists between the plates marked  $\tau$  in both orders, either in the older or in the less developed stages. The anomalous position which would arise from regarding the terminal of the Ophiurid ray as homologous with the first radial of the Crinoid calyx has already been discussed; and the arguments adduced in the case of the Ophiurid apply with equal, if not greater, force in the case of the Asterid. If the terminals in the two groups are not homologous elements, which plates in the one group would represent these plates (the terminals) in the other? Surely no one would maintain that the terminal in the Asterid ( $\tau$  in fig. 14) is homologous with the first radial in the Ophiurid (4 in fig. 12). Or that the terminal of the Ophiurid ( $\tau$  in fig. 12) was homologous with the first radial in the Asterid (4 in fig. 14). No other conclusions would be possible if the homologies I have indicated be denied; and the acceptance of either of these contradictory assumptions would land us into an anomalous position, the untenability of which is obvious.

The comparatively large size of the terminal plate at an early stage of the young Asterid is due, in my opinion, to the coalescence of primitive lateral plates with the primitive or first formed rudiment of the terminal—a circumstance which further strengthens my view of the secondary character of the terminal plate. In like manner I would point out that the series of plates (brachials), which extend along the median dorsal line of the ray, diminish in size and age as they proceed outward from the plate which I have spoken of as the first radial.

Turning now to the actinal surface, we find in the young Starfish at the stage given in fig. 14, that the plate called by



Viguier<sup>1</sup> the odontophore, has almost, if not quite, attained its ultimate position. On studying a series of the earlier stages, we learn that this plate has followed, during the process of development, a path similar to that taken by the mouth-shield of the Ophiuran, with which I consider it to be homologous, and consequently homologous with the oral plate of the Crinoid, as previously held by Ludwig<sup>2</sup> and still maintained by Carpenter,<sup>3</sup> although Ludwig<sup>4</sup> has altered his opinion. I shall presently revert to this subject and mention other points in support of the same view.

The madreporite or water-pore in Asterids usually punctures a basal plate (3 in fig. 14), although in some species it permanently lies external to this plate; and in the early stages of even those in which the basal plate is actually punctured the pore is probably always more or less marginal and is probably at first quite disconnected from the plate.

In the earlier stages of the Asterid larva—and I would here refer with admiration to the careful and most excellent memoir of Ludwig<sup>5</sup>—the first-formed plates in the viviparous or abbreviated larva are the primitive elements of the terminal plates, the basal plates, and the dorsocentral plate. These become well-developed before any traces of the radial plates make their appearance. Concurrent with the radial plates the lateral plates (interambulacral plates) are developed. The diagram given in fig. 15 represents this stage of growth.

Referring to the splendid and now classical memoir of A. Agassiz<sup>6</sup> on the "Embryology of Echinoderms," we find a similar order of appearance of plates obtaining in Asterids which pass through a brachiolarian stage; and indications of

<sup>1</sup> "Anatomie comparée du squelette des Stellérides," 'Archives de Zool. expér. et gén.,' t. vii, p. 33—250.

<sup>2</sup> 'Morphol. Studien,' i, p. 263; ii, pp. 3, 16.

<sup>3</sup> "Notes, v," 'Quart. Journ. Micr. Sci.,' vol. xxii.

<sup>4</sup> "Entwickl. der *Asterina gibbosa*," 'Morphol. Studien,' ii, p. 181.

<sup>5</sup> "Entwicklungsgeschichte der *Asterina gibbosa*," 'Zeitschr. f. wissensch. Zool.,' Bd. xxxvii; ('Morphol. Studien,' Bd. ii).

<sup>6</sup> 'Contrib. Nat. Hist. U.S.,' vol. v.

the same order of development may also be traced, to a certain extent, in the earlier works of Müller<sup>1</sup> and Metschnikoff.<sup>2</sup>

The plates which I have considered to be first radials (fig. 15; 4, 4), are called "intermediate plates" by Ludwig,<sup>3</sup> as he considers the terminal plate homologous with the first radial of a Crinoid; and it will be seen that I also differ from my friend Carpenter in the lettering of his fig. 4 in his last paper, No. 5, of Notes on Echinoderm Morphology.<sup>4</sup> In this Carpenter had followed Ludwig, and it is not difficult to understand how such a misconception of this plate, the radial, should have arisen when only the very early phases of the larva are taken into consideration, without reference to those of fuller growth. Carpenter has candidly admitted the justice of my view and has given expression to the same opinion in his accompanying paper (ante, p. 3).

Having now briefly discussed the homologies of the plates in the larvæ of well known species, which have been carefully observed by previous writers, I would direct attention to an interesting deep-water Starfish which affords a striking confirmation of the views I have expressed; and it is further important to note that in this case, the plates of which we have been treating maintain their distinctness and character throughout life, and do not become concealed or masked in the adult stage, as happens in the forms we have been considering. Of the Starfish in question, *Zoroaster fulgens*, Wyv. Thomson, I have had the opportunity of studying a series of specimens in various stages of growth, obtained by the "Porcupine" Expedition, and more recently by Mr. John Murray during the cruise of H.M.S. "Triton"<sup>5</sup> in the Faerøe Channel. The young *Zoroaster fulgens* has a very remarkable appearance owing to the prominence and distinctness of the component

<sup>1</sup> 'Ueb. die Larven u. die Metamorphose der Echinodermen,' Abhandl. iv (1852).

<sup>2</sup> "Studien ueb. die Entwicklung der Echinodermen u. Nemertinen," Mem. Acad. St. Petersburg; T. xiv (1869).

<sup>3</sup> Op. cit., p. 180, Taf. viii, figs. 99 and 106, *Jm.*

<sup>4</sup> Op. cit., p. 12, fig. iv.

<sup>5</sup> 'Trans. Roy. Soc. Edin.,' vol. xxxii.

plates of the skeleton, and has an unmistakable crinoidal facies, and is highly suggestive of the singular genus *Ophiopyrgus* amongst Ophiurids. The disk is proportionately much higher than in the adult. The dorsocentral plate (fig. 16; 1) is prominent and assumes the shape of a rounded cone. It is surrounded by a circle of five interradially placed plates (3, 3), homologous with the basals of a Crinoid, and these are succeeded by an outer series of five radially-placed plates (4, 4), which alternate with them and are homologous with the first radials of the Crinoid.

These basal and first radial plates are of nearly equal size, very tumid and almost semiglobular in form. They fit close together and occupy by far the greater part of the abactinal area of the disk. The terminal (ocular) plates ( $\tau$ ,  $\tau$ ) at the extremity of the rays are large, somewhat resembling the shape of a serpent's head, and are armed near the extremity with one or two pairs of comparatively large robust spinelets which are directed outwards. The plates of the median dorsal line are large and distinct, and occupy a large portion of the abactinal surface of the ray. The so-called dorso-lateral series of plates form the margin of the ray, and the intermediate plates are small. Between the "dorso-lateral" series and the adambulacral plates there are not more than two fully developed longitudinal rows of plates, with a partially developed series commencing to appear between the latter and the adambulacral plates. The madreporiform body is outside and external to the interradiial plate, and almost in the ravine of the interbrachial angle. The anal aperture is excentric, situated between the dorsocentral plate and an interradiial (i. e. basal) plate, and stands in the right posterior interradius when the madreporiform body is placed in the right anterior interradius.

In fully-grown specimens with rays 125—130 millim. long, the plates above enumerated maintain all their characteristics, and are in no way masked. They have increased greatly in size, and have likewise become modified in shape. The first radials 4, 4, are much larger than the terminals  $\tau$ ,  $\tau$ ; and had this been the form generally studied for the investigation of

the homologies of plates instead of larvæ at very early stages, it is hardly to be imagined that any one would have thought of suggesting that the terminal plate should be taken as the homologue of the Crinoid first radial.

In sequence to the foregoing remarks, it may be stated that the number of Starfishes which maintain these primitive plates (as they may be called) throughout life, and in which the homologies of the crinoidal plates can be studied without having recourse to the larval stages, is much greater than has perhaps been hitherto supposed. It is unnecessary for the present paper to enter into a detailed recapitulation of the different forms by name, and it will be sufficient to say that numerous species comprised in the genera *Pentagonaster*, *Tosia*, *Astrogonium*, *Stellaster*, *Nectria*, *Ferdina*, *Pentaceros*, *Gymnasteria*, and even some in *Scytaster* and *Ophidiaster* may be referred to.

Mr. Carpenter's work on the Ophiurids in a former page, with a similar result, has already been spoken of. The points which he has brought to light are of the most interesting description.

An important feature presented by the larger larvæ and the fully-grown examples of *Zoroaster fulgens* consists in the presence of well-developed under-basal plates (fig. 16, 2, 2), which form a circle round the dorsocentral plate internal to the circle of basal plates and radially disposed. These plates are homologous with the under-basals of dicyclic Crinoids (e.g. *Cyathocrinus*, *Encrinus*, and *Marsupites*), and are marked 2, 2 in Carpenter's nomenclature. The presence of under-basal plates is apparently not a rare occurrence in Asteroids; and it may here be remarked that the representatives of these plates are developed in larval *Asterina gibbosa*, at a stage later than that studied by Ludwig<sup>1</sup> (see fig. 14; 2, and that similar plates which I likewise homologate with under-basals are to be found in young tests of *Asterias rubens* and *A. glacialis*. They are large and well developed in the adult forms in *Pentagonaster semilunatus*, *Gym-*

<sup>1</sup> 'Zeitschr. f. wissensch. Zool.,' Bd. xxxvii; ('Morphol. Studien,' Bd. ii).



*nasteria carinifera*, in various species of *Pentaceros*, and a large number of the *Goniasteridæ*.

The presence of under-basals in Asterids and Ophiurids (first pointed out in the latter group by Carpenter<sup>1</sup>) apart altogether from their presence in numerous Crinoids, goes very strongly, in my opinion, against the views of A. Agassiz, Lovén, Wachsmuth, and other writers, who consider that the dorsocentral plate of Echinoderms is the solidified homologue of the five under-basals (or basals of earlier writers) of a Crinoid; and that a support which almost amounts to confirmation is given to Carpenter's<sup>2</sup> opinion that the dorsocentral plate, which is simple from its earliest embryonic commencement, is indeed the homologue of a single and simple plate. This plate Carpenter<sup>3</sup> homologates with the radical plate or root-disk at the extremity of the Crinoid stem. This view I endorse entirely, and fail to see how five plates, even when ankylosed, can be homologated with an embryonically single and simple plate.

It seems to me that Ludwig places too much importance upon the puncture of the water-pore (the madreporite) as furnishing the index for determining the homologies of plates.<sup>4</sup> Because the oral plate in Crinoids bears this puncture, the presence of the water-pore in any plate whatever is held by him as indicating that the plate thus furnished is the homologue of the oral plate of the Crinoid, irrespective altogether of whether the plate be developed on the right or the left peritoneal diverticulum—a question undoubtedly of far greater morphological importance.

All that we know about the early embryonic stages of Starfishes goes to show that primarily the water-pore is independent from any plate whatever, and that it is only at a later period that it becomes engulfed as it were, or surrounded, by the extensions

<sup>1</sup> 'Notes on Echinoderm Morphology,' No. v, p. 10 (separate copy).

<sup>2</sup> "Oral and Apical Systems of Echinoderms," 'Quart. Journ. Micr. Sci.,' vol. xviii, p. 371, et seq.

<sup>3</sup> Op. cit., p. 374.

<sup>4</sup> Cf. Carpenter, 'Quart. Journ. Micr. Sci.,' vol. xx, p. 322; also F. M. Balfour, 'Comparative Embryology,' vol. i, p. 477 (note).

of the rapidly growing adjacent basal plate. Even when the Asterid has attained maturity the position of the water-pore (*i. e.*, the madreporite) is more or less lateral in its position on the plate, a fact which has been observed by Ludwig<sup>1</sup>, who also remarks on the similar position of the water-pore in relation to the punctured plate both in Ophiurids and Crinoids.

These points alone are sufficient to indicate that the relation of the water-pore to the plate which may be associated with it is not of such an intimate nature as has generally been supposed; and it is still more conclusive to find that in a large number of Starfishes the madreporite, even in the adult form, is situated external to, and often independent and well separated from, the basal plate. Without enumerating cases in detail it will suffice to mention species of *Pentaceros*, *Astrogonium*, *Tosia*, *Pentagonaster*, *Stellaster*, *Goniodiscus*, *Ferdina* (?); together with *Gymnasteria carinifera*, *Dermasterias imbricata* (fide Gray), *Scytaster variolatus* (? part), *Ophidiaster pyramidatus* (fide Viguiet), and *Zoroaster fulgens*. The total disconnection of the madreporite from the oral plates in the *Astrophytidæ* (*Astrophyton*, *Trichaster*, &c.) may likewise be referred to as supporting the same view.

These circumstances unquestionably prove that the position of the water-pore is not in a definite and unchangeable relation to a given plate and that its presence per se is not a reliable index of the homology of that plate. Furthermore, they strengthen the view that morphologically the position of the water-pore is a travelling factor and may occupy any position upon the interradial line—an argument that derives further support from what is known of the embryology of the group.

It may also be mentioned incidentally here that a similar travelling position along the interradial line has already been shown by A. Agassiz<sup>2</sup> to occur in the phylogenetic history of the periproctal aperture in the Echinoids.

Trusting upon the supposed homology of the plates punctured

<sup>1</sup> 'Entwickl. Ast. gibb.,' p. 160.

<sup>2</sup> 'Bull. Mus. Comp. Zoöl.,' 1869, ser. 3, No. 9, p. 295.

tured by the water-pore, Ludwig has been led to consider that the ventral area of the Crinoid is comparable to the dorsal area of Asterids and Echinoids. This view has been ably controverted by Carpenter<sup>1</sup> from the Crinoid standpoint; and it is only necessary to remark here that if there be any validity whatever in the homologies of calyx plates, which have been considered in the preceding pages, as occurring in Asterids and Ophiurids, the supposition of Ludwig's must fall to the ground. In the case of the Echinoids Ludwig's theory<sup>2</sup> is to my mind altogether untenable, for the genital plates at the apical pole are homologated to the oral plates of the Crinoid, notwithstanding that the larval central plate is regarded by him as the homologue of the dorsocentral plate of the group or the root-disk at the end of the Crinoid stem, as maintained by Carpenter.

A further outcome of this hypothesis is that the basal plates of Crinoids have no representatives in the Echinoid test. It is hard to imagine a more anomalous position than that in which plates developed on the left peritoneal diverticulum of Crinoids (the orals) are homologated with plates developed on the right peritoneal diverticulum of Echinoids (the genitals) and made to surround the homologue of the plate (the dorsocentral), which in the Crinoid is unquestionably developed at the end of the right peritoneal diverticulum. The fact that the basal plates of the Crinoid, the Asterid, the Ophiurid and the Echinoid are all developed on the right peritoneal diverticulum or enterocœl and that the oral plates and their representatives are, on the other hand, all primitively formed on the left enterocœl, is, in my opinion, conclusive evidence that the dorsal or abactinal area of the Crinoid is comparable with the dorsal area of the Asterid, the Ophiurid and the Echinoid, as Carpenter<sup>3</sup> has justly maintained.

Before leaving the subject of the madreporite I would here add a few remarks on the orientation of the Echinoderm larvæ

<sup>1</sup> "Notes on Echin. Morphology," No. v, p. 12, 'Quart. Journ. Micr. Sci.,' vol. xxii.

<sup>2</sup> 'Morphol. Studien,' Bd. ii, pp. 42, 43.

<sup>3</sup> 'Notes, v,' p. 16.

in general. It may be said to be universally admitted that the interradius with which the water-pore or madreporite is associated stands in a definite and fixed relation to the internal organisation. This relation is constant throughout the group. It is natural, therefore, that the madreporite has always been regarded as an important superficial index, whereby the orientation of an Echinoderm may be effected. Taking these statements as a premiss, I would now refer to Lovén's classical memoir on the structure of the Echinoidea,<sup>1</sup> in which the bilateral symmetry of all Echinoids, both regular and irregular, is most clearly established. The madreporite is here shown to be invariably associated with the right anterior interradius, when the test is viewed from above. If Lovén's demonstrations are accepted as conclusive (and I myself admit them and consider that the evidence in its general bearings is as yet unshaken<sup>2</sup>), it necessarily follows that as the position of the madreporite is fixed and definite in its relation to the internal anatomy of the organism, and consequently to the antero-posterior axis, throughout all the group, the axis of bilateral symmetry is the same in each, and the madreporite holds the same definite relation thereto as predicated above. On these grounds I regard the interradius of the madreporite or water-pore of the Crinoid, the Ophiurid, and the Asterid, as the right anterior one when viewed from the dorsal surface; and in conformity with this opinion I have thus placed the diagrams of the respective larvæ referred to in the preceding pages. It seems unnecessary that I should here enumerate the various diverse views which have been held by other writers upon the subject.

Whilst treating of the madreporite I am naturally led to remark on a recent paper by my friend M. Perrier<sup>3</sup> on

<sup>1</sup> "Études sur les Échinoïdées," 'Kongl. Vet. Akad. Handl.,' Bd. xi, No. 7.

<sup>2</sup> Although I have very carefully studied Prof. A. Agassiz' discussion of this question in his 'Revision of the Echini' and in his 'Report on the "Challenger" Echinoidea,' I do not yet feel disposed to relinquish my adhesion to the opinions above expressed, in so far as the significance of the test structures, when taken as a whole, is concerned.

<sup>3</sup> 'Comptes Rendus,' 10 July, 1882, t. xev, pp. 61-63.



*Brisinga*, in which the madreporiform body is stated to be always formed on one of the odontophores in that Starfish. From this the conclusion is drawn that *Brisinga* occupies an intermediate position between Asterids and Ophiurids. With this statement I am unable to agree, as I do not find when a section is made of the disk of *Brisinga* any connection whatever between the madreporite and the odontophore, the former being merely superposed over the odontophore in consequence of its own extreme position at the margin of the disk, and the prominence and size of the odontophore. No morphological relation exists whatever. The origin and mode of formation of the odontophores in *Brisinga* which M. Perrier describes are very remarkable. They are described as formed from the plates of the proximal ring of interrarial plates which originally surround the dorsocentral plate, that is to say, from the plates which we have designated basals (marked 3, 3) in the preceding pages. "Les pièces interbrachiales sont ainsi refoulées constamment vers le bord du disque; en même temps, elles se réduisent de plus en plus, viennent se placer exactement dans l'angle des bras, cessent ainsi peu à peu de faire partie du squelette du disque et finissent par constituer les odontophores. Ainsi les odontophores sont les restes des pièces du premier rang du disque primitif de la *Brisinga*."<sup>1</sup>

Arguing from the identity of the plan of organisation in *Brisinga* and the true Asterids, Perrier concludes that the odontophores are formed in a similar manner in the Starfishes generally. From this conclusion I dissent entirely, for in none of the Starfishes of which we know anything of the embryological stages does such a transformation of basals into odontophores take place. The two plates are separate and distinct, and co-exist independently from their first formation. In further support of this remark, I need only refer to the large number of forms, briefly indicated in a preceding page, in which the primitive interrarial or basal plate remains prominent and distinct even in the adult form. Furthermore, believing as I do that the odontophore has its development in

<sup>1</sup> l. c., p. 63.

the actinal hemisome, and consequently formed on the left enterocœl, one might almost be so bold as to assert that the method of origin suggested by Perrier was theoretically impossible, involving as it does the assumption that a plate primitively located on the abactinal hemisome, and developed upon the right enterocœl should, during the process of growth, pass away from that position to become metamorphosed into, and assume the form, position, and functions of, a plate belonging to the actinal hemisome and developed upon the left enterocœl, which normally exists independently. As I have not had the opportunity of examining any examples of *Brisinga* as young as those which Perrier has studied, I am unable to speak personally of the phases of the development of the plates in question in that form; but I trust that M. Perrier will acquit me of discourteous scepticism if I suggest on the theoretic grounds above enunciated, that possibly some misinterpretation may have arisen, owing to the intricacy of the subject and the difficulty of investigating such a rare organism in its earliest stages.

I have previously stated my belief that the odontophores of Asterids are homologous with the mouth-shields of Ophiurids and the orals of Crinoids. This view was originally held by Ludwig,<sup>1</sup> but has subsequently been rejected by him<sup>2</sup> in favour of their recognition as odd interambulacral plates homologous with the "plaques peristomiennes" of Lovén<sup>3</sup> in Spatangoids and Clypeastroids. From the embryological standpoint I am still disposed to maintain Ludwig's previous position. It is true that the homology between the odontophore of the Asterid and the mouth-shield of the Ophiurid is at first sight somewhat obscured by the difference of position; the mouth-shield being a superficial plate externally visible on the actinal surface, whilst the odontophore of Asterids is usually situated at a much deeper level and is masked by the mouth-plates. This con-

<sup>1</sup> 'Morphol. Studien,' Bd. i, p. 263; Bd. ii, p. 3.

<sup>2</sup> 'Morphol. Studien,' Bd. ii, p. 181.

<sup>3</sup> 'Études sur les Échinoïdées,' 'Kongl. Svenska Vet. Akad. Handl.,' Bd. ii, No. 7.

cealment, however, is only a question of the special development of the last-mentioned plates (i. e. modified adambulacrals) in Asterids as compared with Ophiurids, for when some of the deep-water Starfishes are examined, with which we have recently become acquainted (e.g. species of *Porcellanaster*, *Styracaster*, *Hyphalaster*, and *Thoracaster*), we find forms in which this difference is by no means so great, a part at least of the odontophore being superficially visible on the actinal surface.

In conclusion, it may not be out of place to compare very briefly inter se the characters of the earliest stages of the development of the brachiata Echinoderms. Noting, in the first place, for the whole group generally, that a more or less definite centrifugal movement of plates takes place during growth, and that this is specially manifest in the phylogenetic history of the group, we may proceed to indicate two natural sets of plate-developments, viz. one defined by the basal plates—a basi-oral or interradiat series; and another defined by the radial plates—a radio-terminal or radial series; the proximal or adcentral factors in each series being more or less stationary.

In the earliest stages of the Crinoid it has already been observed that the basi-oral series primarily constitutes the whole calyx, the radial series being of a later or retarded development. During the process of growth (at least in the form under notice) the radial series develops with disproportionate rapidity, and at a comparatively early and still premature stage comes to predominate over the basal series.

In the Ophiurid, on the other hand, we are presented with an accelerated radial development, the radials being first-formed, and the basal series not appearing till later.

In the Asterid we have a retarded radial development as in Crinoids, the basals being first formed, and the radial series being of later growth.

It is interesting to note that in both the Asterid and the Ophiurid the outer or distal plate of the retarded series appears earlier than the inner or adcentral factor; the oral plates in

Ophiurids being developed before the basals, and in Asterids the terminals are formed before the radials.

In both Asterid and Ophiurid the representatives of the under-basals do not appear until the other plates are well developed.

The occurrence of under-basals in the Asterid as well as in the Ophiurid larvæ nullifies the argument advanced by Studer<sup>1</sup> that the arrangement of primitive plates in Asterids and Echinoids corresponds with the monocyclic Crinoids, whilst that of Ophiurids corresponds with the dicyelic Crinoids.

It is obviously premature to construct reliable hypotheses solely upon the basis of the comparisons above instituted. Our information is still too limited; and an extended series of observations upon the developmental stages of a larger number of forms in each group of the Echinodermata is necessary before the validity of any deductions can be assured. The facts, however, above enumerated are very significant and suggest at least the conclusion that the Ophiurid derives its origin from a more highly developed Crinoid ancestor than the Asterid, whose characters it has also retained more preponderately; that the Asterid was in all probability derived from a Crinoid of more primitive type; and that the two forms advanced along collateral lines of descent. Upon the question of the priority of origin, it is still premature to invoke embryological evidence.

<sup>1</sup> "Uebers. ueb. die Ophiuriden welche während der Reise S. M. S. Gazelle gesammelt wurden," p. 10, 'Abhandl. d. Königl. Preuss. Akad. d. Wissensch. zu Berlin, vom Jahre 1882.'



## On the Origin of Metameric Segmentation and some other Morphological Questions.

By

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With Plates II and III.

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IN the following pages<sup>1</sup> certain hypotheses with regard to the evolution of segmented Triploblastica (Annelida, Arthropoda, Vertebrata), and some apparently unsegmented forms (Mollusca Brachiopoda, Sagitta, Balanoglossus) are suggested and discussed.

I have found it convenient to consider the Vertebrata specially in the latter part of the paper, because of the very pronounced views which are held at the present day with regard to their evolution.

The paper is divided into two parts. The first part deals with the evolution of certain organs; the second part with the evolution of the groups mentioned and especially with that of the Vertebrata.

My hypothesis concerning the origin of metameric segmentation has been in a sense anticipated by Lang. He regards the somites as derived from gut pouches such as are found at the present day in Turbellarians. It should be remembered that according to his view, the Turbellaria are specialised Coelenterates. My view of the origin of Somites differs from

<sup>1</sup> A short account of the main points of this paper was communicated to the Cambridge Philosophical Society in November, 1883, and published in vol. v of the 'Proceedings' of that Society.

his in taking a simpler diploblastic form as the starting point for all the Triploblastica discussed.

Hubrecht in his recent paper on the "Ancestral form of the Chordata" has explained Lang's views and instituted some important comparisons between Vertebrates and Nemertines. I differ, however, from Hubrecht in taking a simpler form as my starting point.

I have purposely refrained from referring to the Turbellaria and other flat worms in this essay, because I cannot make up my mind as to whether they are degenerate Enterocœla or highly specialised Cœlenterata (without a separated cœlom). I am, however, very much inclined to the view that they are degenerate Enterocœla.

I have also avoided discussing the Echinoderms because, while their early development is easy to understand, the later stages and metamorphosis are not so intelligible.

My hypothesis with regard to the origin of the mouth and anus has, so far as I know, not been suggested before. I agree with Hæckel in regarding the blastopore as homologous with the primitive mouth of the gastræa.

I have attempted to explain the peculiar behaviour of the blastopore in a general way, in the first part of my paper. In the second part I again consider this question in connection with the Vertebrate blastopore. I dissent most strongly from the view that the Vertebrate mouth and anus are both secondary perforations, and not homologous with those of Invertebrates, e. g. Annelids. I regard them both as homologous with the corresponding structures in the other Triploblastica discussed.

But I have not been able to do justice to this part of my subject. I could only do so by reviewing critically the extensive literature on this subject, and by making a special investigation of the behaviour of the blastopore in animals with a prolonged larval life, and of the structures classed as primitive streaks, and this I have unfortunately been unable to do. I think that any such investigation would have valuable results.

I agree with Balfour in his view that the "concrecence"

theory of the growth of the Vertebrate embryo is untenable. It seems to me that the advocates of that theory have mixed up three distinct embryonic structures, the mesoblastic bands, the primitive streak, and the ridges of the medullary groove.

The primitive streak is in most forms at first a median structure. I agree with the current view as to its nature as a rudiment of the blastopore, and I suggest a reason for its persistence.

I ought particularly to mention that I regard the Annelid-origin of the Vertebrata and Arthropoda as untenable. This will be obvious to anyone reading the following pages.

I offer no suggestion as to the phylogeny of Mesoblast. I agree entirely with the current view that it has arisen from both of the primary layers.

Mesenchyme is obviously merely precociously developing mesoderm, and is particularly developed in free larvæ.

Finally, I may add that I do not put forward these hypotheses in a dogmatic spirit, and that I fully recognise that theories dealing with the complicated facts of morphology can only have in most cases a very temporary value. The main idea of the comparisons discussed below first occurred to me some years ago, when investigating the development of the Vertebrate excretory organs; but they have received such striking confirmation from Hatschek's work on *Amphioxus*, and more recently from a study of the embryo of *Peripatus capensis*, that I have at length decided to publish them, hoping that they may at least excite criticism and so lead to the increase of our knowledge, and to the greater definition of our ideas.

In the discussion which followed the communication of the late Professor Balfour's notes and drawings of the early embryos of *Peripatus Capensis*, to the Royal Society (December, 1882), I drew attention to the great resemblance between the embryo of *P. Capensis* with its elongated blastopore and somites, and an adult *Actinozoid* polyp. I pointed out that the comparison of these two structures suggested an explanation, which so far as I know has not before

been suggested, of a great morphological difficulty, viz. the origin of metameric segmentation (vide 'Nature,' December 28th, 1882). At the same time I pointed out that by following up this comparison some other morphological difficulties received an explanation.<sup>1</sup>

The hypotheses I suggested were shortly as follows :

1. The mouth and anus found in most of the higher groups (Vermes, Mollusca, Arthropoda and in all probability Vertebrata) have been derived from the mouth of an ancestor common to them and the Cœlenterata; i.e. from an elongated opening such as is found at the present day in the Actinozoa.

2. That the somites of segmented animals are derived from a series of pouches of the primitive gut (archenteron) of a Cœlenterate-like ancestor, i.e. from pouches generally resembling those found at the present day in Actinozoid polyps and Medusæ.

That the excretory organs or nephridia (segmental organs) of the higher animals are derived from specialised parts of these pouches which were in the supposed ancestor, as indeed they now are in many living Medusæ and Actinozoid Polyps connected peripherally with each other by a longitudinal canal (circular canal of Medusæ, perforations in mesenteries of Actinozoa,) and with the exterior by a pore<sup>2</sup> one for each pouch; further, that in the Invertebrata, e.g. Annelida, the longitudinal canal has been lost and the external pores retained, while in the Vertebrata the longitudinal canal (segmental or pronephric duct) has persisted and

<sup>1</sup> Mr. E. B. Wilson, who was present when this discussion took place at the Royal Society, and to whom I subsequently at Cambridge showed the specimens and drawings of the *Peripatus* embryo, informs me that the work (referring to Polyps) which he has since done at Naples has enabled him to give some additional evidence in favour of my views. As Mr. Wilson's observations are not yet published, I am unable to quote them here; but he informs me that his paper is in the press, and will shortly appear in the Naples 'Mittheilungen.'

<sup>2</sup> Vide Hertwig, 'Organismus der Medusen,' p. 39; and "Actinien," 'Jena Zeitschrift,' Bd. xiii.



retained its posterior opening into the alimentary canal while the external pores have been lost.

I now add to these three propositions a fourth.

4. That the tracheæ are not developed from cutaneous glands of a worm-like animal with well differentiated mesodermal tissues (a view which on physiological grounds is hard to accept) but are rather to be traced back to simple ectodermal pits in the two-layered ancestor developed for purposes probably of aeration and represented at the present day in the Cœlenterata by the subgenital pits of the Scypho-medusæ, in the embryos of Arthropoda by the pits into the cephalic ganglia, and in the Vertebrata by the canal of the central nervous system.<sup>1</sup>

The essence of all these propositions lies in the fact that the segmented animals are traced back not to a triploblastic unsegmented ancestor but to a two-layered Cœlenterate-like animal with a pouched gut, the pouching having arisen as a result of the necessity for an increase in the extent of the vegetative surfaces in a rapidly enlarging animal (for circulation and nutrition).

The hypotheses are based upon the embryonic development of the respective organs in the Triploblastica and the structure of the living Cœlenterata; in other words, upon facts precisely of the same nature as those which have been used in tracing the evolution of the nervous and muscular tissues.

Before proceeding to discuss the facts upon which the hypotheses rest, I may be permitted again to point out that it is no part of my view to derive segmented animals direct from the Cœlenterata, but to derive both Cœlenterata and segmented animals from a common Cœlenterate-like ancestor, whose structure can only be elucidated by studying the anatomy and the development of the living Cœlenterates, and of the higher segmented animals.

<sup>1</sup> Sedgwick, "On the Original Function of the Canal of the Central Nervous System of the Vertebrata," 'Proc. of Cambridge Phil. Soc.,' vol. iv.

# ON THE HOMOLOGY OF THE MOUTH AND ANUS WITH THE MOUTH OF THE CœLENTERATA.

It will be generally admitted that the mouth and anus of the Annelida, Arthropoda, and Mollusca are homologous structures—i. e. that the mouth of an Arthropod is homologous with that of an Annelid, and with that of a Mollusc, and that the anus in each of these groups is homologous with the anus of the other groups. It is well known that the blastopore in these groups presents considerable differences in its relation to the mouth and anus. In one form it is directly converted into the mouth, in another into the anus; while sometimes it entirely closes up and gives rise to neither (for summary of facts vide Balfour 'Comp. Embryology,' vol. ii, pp. 281, 282). This variability, in the fate of the blastopore was first pointed out by Lankester.<sup>1</sup> It is very puzzling, and has led some morphologists to regard it as a structure which is not homologous in the different animals, and of no particular phylogenetic significance. It seems to me, however, that a little consideration shows that this view of the blastopore must be given up, and that there are very strong grounds for regarding the blastopore as homologous in every case,<sup>2</sup> and also as homologous with the mouth of the Cœlenterata. Before proceeding to discuss the main point of this section of my paper, I must definitely examine this question about the blastopore.

**On the Blastopore.**—Either the blastopore has an ancestral meaning or it has not. It seems to me that we have no right to assume that this or any other embryonic structure or process is without a phylogenetic significance until all other views have been shown to be untenable.

It is often said when any peculiar embryonic process is discussed

<sup>1</sup> "On the Coincidence of the Blastopore and Anus in *Paludina*." This Journal, 1876.

<sup>2</sup> It must be distinctly understood that the only groups referred to in the following paper are the Vertebrata, Annelida, Arthropoda, Mollusca, Balanoglossus, Brachiopoda and Sagitta. For the present, I leave the Platyelminthes and Echinoderms out of consideration. The special case of the Vertebrata will be considered in Part II.

from a phylogenetic standpoint that it is only the way in which the animal develops, and that it is waste of time to attempt to explain it. I cannot agree to accept such a view of any embryonic fact. If there is anything in the theory of evolution, every change in the embryo must have had a counterpart in the history of the race, and it is our business as morphologists to find it out.

I wish to point out that I am not discussing how the gastrula arose. I take as my basis the undoubted fact that gastrulæ have existed, and I am trying to show that a two-layered, gastrula-like animal was the ancestor of most living Metazoa.

I must, therefore, reject the view that the blastopore has no ancestral meaning.

What, then, is its ancestral meaning?

It seems to me that there is very strong evidence for the view that it is homologous with the mouth of the Cœlenterata.

In the first place the Cœlenterate mouth either arises as a result of invagination, the blastopore remaining as the mouth (Cereanthus, Pelagia), or as the result of perforation. In the Triploblastica similarly the blastopore either arises as a result of invagination or as a perforation. The method of development, therefore, coincides, and we thus have a strong reason for regarding them as homologous.

The second important point to be examined in determining homologies is the relation to other important structures. The relation of the Cœlenterate mouth and the blastopore to the alimentary canal and the nervous system can in most cases be determined; and in all cases in which it can be so determined, it is the same.

(1) The Cœlenterate mouth and the blastopore resemble each other in being the main communication by which the archenteric cavity or its rudiment communicates with the exterior.

(2) They resemble each other in always being perforations of the neural surface of the body.

With regard to the first of these agreements nothing need be said; it is a fact of little importance, as there are many other channels in the Cœlenterata through which the archenteron communicates with the outer world. The second agree-

ment is of great importance; but before it can be of any value to us, we must be able to decide whether the neural surfaces of the Cœlenterata, Annelida, &c., are homologous. It will be generally admitted that the nervous systems of the Annelida, Arthropoda and Mollusca are built upon the same type; and that the ventral surface of the body is homologous in each of these three groups. The late Prof. Balfour put forward the hypothesis that the nervous system of these types was homologous with that of Cœlenterata. He says:

“In the first place it is to be noted that the above speculations render it probable that the type of nervous system from which that found in the adults of the Echinodermata, Platyelminthes, Chætopoda, Mollusca, &c., is derived, was a circumoral ring, like that of Medusæ, with which radially-arranged sense-organs may have been connected; . . . . Its anterior part may have given rise to supra-œsophageal ganglia and organs of vision; these being developed on the assumption of a bilaterally symmetrical form, and the consequent necessity arising for the sense-organs to be situated at the anterior end of the body. If this view is correct, the question presents itself as to how far the posterior part of the nervous system of the Bilateralia can be regarded as derived from the primitive radiate ring.

“A circumoral nerve-ring, if longitudinally extended, might give rise to a pair of nerve-cords united in front and behind, —exactly such a nervous system, in fact, as is present in many Nemertines (the Enopla and Pelagonemertes), in Peripatus and in primitive molluscan types (Chiton, Fissurella, &c.). From the lateral parts of this ring it would be easy to derive the ventral cord of the Chætopoda and Arthropoda. It is especially deserving of notice, in connexion with the nervous system of the above-mentioned Nemertines and Peripatus, that the commissure connecting the two nerve-cords behind is placed on the dorsal side of the intestines. As is at once obvious, by referring to the diagram (fig. 231 B), this is the position this commissure ought, undoubtedly, to occupy if derived from part of a nerve-ring which originally followed more or less closely the



ciliated edge of the body of the supposed radiate ancestor." 'Comparative Embryology,' vol. ii, pp. 311, 312.

It seems to me that nothing can be added to make the case stronger. I only wish to make one addition to the hypothesis, and that is that the type of nervous system from which that of the above-mentioned groups has been derived was a broad ring round the mouth, in fact, more resembled the nervous system of *Actinia* in its general diffusion over the oral surface than the compact ring of the *Medusa*; the latter being a highly specialised part of this generalised nervous system, which has, however, in part persisted in the subumbrella plexus of ganglion cells described by Schafer and Claus. If this hypothesis is correct, i. e. if it be true that the oral surface of a *Cœlenterate* is homologous with the ventral surface of the mentioned groups; and if the nerve-ring of the *Medusa*, the nerve-ring of *Peripatus*, the nerve-ring and general ventral nervous plexus of *Chiton* and *Proneomenia*, the cerebral ganglia and ventral nerve-cords of other *Mollusca*<sup>1</sup> and *Annelida* and *Arthropoda* are all derived from a general peri-oral nervous system of a *Cœlenterate*-like ancestor, then the relation of the blastopore to the nervous system is the same in the *Annelida*, *Arthropoda* and *Mollusca* and the same as that of the mouth of *Cœlenterata*.

With these facts before us, viz. similarity in development and in relation to other important structures, I think we can hardly doubt the fact that the blastopore in the cases mentioned and the *Cœlenterate* mouth are homologous structures.

In the above discussion I have avoided referring to the ultimate history of the blastopore. The fate of the blastopore in the *Triploblastica* is extremely variable, and it is this variability only which has caused the homology ever to be doubted.

But I think we have here two distinct questions: one deals with the blastopore or mouth of the two-layered stage in

<sup>1</sup> The absence of the connection dorsal to the anus in some *Mollusca*, *Annelida*, and *Arthropoda*, will not I think be regarded as a fact of any importance if the hypothesis be accepted with regard to the nervous system of *Peripatus* and *Chiton*.

embryonic development and asks whether that stage has a counterpart in evolution; the other deals with the subsequent development of the blastopore and asks whether that subsequent development throws any light on the evolution of the mouth and anus.

But at the same time I must admit that the fate of the blastopore is so peculiar, that the doubts which on that account have been expressed as to its phylogenetic meaning are not unreasonable. The case stands thus. The blastopore in *Serpula* gives rise to the anus; in most other Chætopoda to the mouth; similarly in the Mollusc *Paludina* it becomes the anus, while the general rule among Mollusca is that it should become the mouth. It would seem to follow from these facts, as Lankester has already pointed out, that if the blastopore is in each case homologous, then the anterior end and mouth of *Serpula* must be homologous with the posterior end and anus of other closely allied Chætopods. This is manifestly absurd. There are two ways out of the difficulty; either the homology of the blastopore must be given up, or we must suppose that primitively it gave rise to both mouth and anus, and that its specialisation as a larval organ has caused the variability of its subsequent history. The latter view is obviously suggested by the elongated form the blastopore first assumes in many animals, extending as a slit along the whole of the ventral surface of the embryo.<sup>1</sup> The blastopore never retains for long this form, but soon becomes specialised to a round opening, the definite blastopore,<sup>2</sup> by the closure of the lip of the slit except at one point. The point at which it remains open must depend on the shape of the larva, &c., and will obviously be determined by the convenience of the larva.

This hypothesis that the mouth and anus of the Triploblastica is derived from a single opening, represented in living animals by the Cœlenterate mouth and, on the assumption

<sup>1</sup> This fact was first pointed out by Lankester, vide this Journal, vol. xvi, 1876, p. 326.

<sup>2</sup> A special name is wanted for this structure, to distinguish it from the blastopore of the gastrula stage.

(vide above p. 49) that the latter and the blastopore of higher types are homologous, by the early blastopore (before specialisation as the larval mouth) receives very strong support from the actual structure of the Actinozoid mouth, and from the newly discovered facts with regard to the history of the blastopore of *Peripatus capensis*; and has the merit of being on a priori physiological grounds easily conceivable.

**Mouth of the Actinozoa.**—In the Actinozoa the mouth-opening is elongated, and the animal is symmetrical on each side of the long axis of the mouth. At one end of the long axis the mouth is especially differentiated, and this differentiation extends down the stomodæum as a strongly ciliated groove called by Hickson<sup>1</sup> the Siphonoglyphe. The cilia of this groove produce a current from without inwards, while the cilia of the rest of the stomodæum work in the opposite direction. This differentiation of the stomodæum is particularly conspicuous in the Hexatinian *Peachia*, in which there is a deep strongly muscular groove along the whole length of one side (the so-called ventral side) of the stomodæum (fig. 6, *Si*); and the walls of the groove project at the mouth-opening beyond the rest of the wall of the stomodæum so as to form a semi-circular lip conspicuous from the exterior at one end of the long axis of the mouth.

The free edges of this groove are frequently united with each other, so that the groove is converted into a canal open into the general cavity of the body at the lower end of the stomodæum, and to the exterior at the mouth-opening. This junction of the lips of the groove seems to be simply a case of adhesion, as they may with very slight effort be separated without tearing the tissue. When the groove is thus converted into a canal there are obviously two openings into the body of the polyp, one through the general opening of the stomodæum, and the other through this highly differentiated siphonoglyphe. According to Hickson (*loc. cit.*) the cilia work in opposite directions in these two parts of the stomo-

<sup>1</sup> 'Proc. Royal Soc.,' 1883.

dæum, so that one may be regarded as a mouth and the other as an anus.

I have not been able to make out what causes the adhesion of the lips of the siphonoglyphe in *Peachia* (whether interlocking of cilia as in *Lamellibranch* gill or what), but of the adhesion there can be no doubt whatever.

This differentiation of the mouth and stomodæum of *Actinozoid* polyps has been known for some time. The Hertwigs,<sup>1</sup> in their brilliant paper on the *Actinozoa*, summarise the facts and point out that the elongated mouth when closed has a dumb-bell shaped form, the median portion being closed, and the two ends remaining open.

“Wenn die Wandungen des Schlundrohrs an einander legt sind und der Mund geschlossen ist, bleiben sie (the ‘Schlundrinnen’) geoffnet und wird demnach ihre Bedeutung wohl darin bestehen, dass durch sie fortwährend ein Wasserstrom in das innere des Körpers hinein getrieben wird” (p. 513).

In view of the hypothesis under consideration, viz. that the mouth and anus of the higher animals is derived from an elongated slit-like opening such as is found in the *Actinozoa*, these anatomical facts are of the highest interest.

**Blastopore of *Peripatus*.**—The history of the blastopore of *Peripatus* has been given up to a certain point in the last volume (1883) of this Journal.<sup>2</sup> The youngest embryo found was a spherical or slightly oval gastrula with a slightly elongated blastopore (fig. 1). In the subsequent growth the embryo becomes elongated along the long axis of the blastopore and the mesoblastic somites appear (fig. 2). The middle portion of the lips of the blastopore then come together (fig. 3), and in the next stage (fig. 4) there are two openings into the mesenteron, an anterior and a posterior. Meanwhile, the primitive streak (connected with the formation of the meso-

<sup>1</sup> “Die Actinien,” ‘Jena Zeitschrift,’ vol. xiii, p. 513.

<sup>2</sup> The species of *Peripatus* which Dr. v. Kennel is working at is different from that described in Balfour’s memoir. Dr. v. Kennel does not mention this somewhat pertinent fact. Perhaps he was not aware of it; but if he was, I find it difficult to understand the positive nature of his criticism.



blast), which was present at the hind end of the blastopore in the earliest stage (fig. 1), has become marked with a groove (fig. 4). In the paper referred to, the question—Do these two openings become the mouth and anus of the adult?—was left open. I am now in a position to state that they do become the mouth and anus of an embryo of an age equal to the oldest stage described by Moseley<sup>1</sup> in his original paper, so that I think there can be no doubt that they do become the mouth and anus of the adult.

Thus, then, we have two undoubted facts :

1. That the mouth of the Actinozoa is differentiated into one portion for the exit and another for the entrance of matter, and that this differentiation is carried so far as to give rise to two separate openings (Peachia).

2. In the development of *Peripatus capensis* the single opening of the gastrula elongates, then divides into two parts, an anterior part which becomes the mouth, and a posterior which becomes the anus of the adult.

The argument may here be briefly summarised :

1. The blastopore of Annelida, Arthropoda, Mollusca, and the mouth of Cœlenterata are homologous because (*a*) of the development (*b*) of the anatomical relations in each case.

2. The structure of the mouth of Actinia and the position of the mouth and anus within the primitive nerve-ring, which is supposed to be homologous with the circumoral nervous diffusion of Actinia, obviously suggests the derivation of the mouth and anus from a single opening like the mouth of Actinia by the completion of the fusion which is there beginning.

3. The blastopore of *Peripatus*, which by hypothesis is homologous with the Cœlenterate mouth and with other blastopores, actually passes through the Actinia phase.

Is this development primitive? If it is primitive, then as the mouth and anus of *Peripatus* are homologous with those of Annelids, my point is gained and we shall have to take the second alternative (p. 52), and suppose that the peculiar

<sup>1</sup> 'Phil. Trans.,' 1874.

behaviour of the blastopore in other cases is due to larval specialisation.

The structure and distribution of *Peripatus* all point to its being an extremely primitive type. We should, therefore, a priori, expect to find that its development showed primitive features.

In the second part of this paper I shall attempt to show that the very variable behaviour of the blastopore is explicable.

It is hardly necessary to point out that the stomodæum and proctodæum are, on the above hypothesis, structures of purely secondary importance, and that I am in agreement with Balfour's suggestion that the stomodæum and proctodæum are not in all cases completely homologous. He says ('Comp. Emb.,' vol. ii, pp. 285, 286), "As a rule an oral and anal section of the alimentary tract—the stomodæum and proctodæum—are derived from the epiblast; but the limits of both these sections are so variable, sometimes even in closely allied forms, that it is difficult to avoid the conclusion that there is a border land between the epiblast and hypoblast, which appears by its development to belong in some forms to the epiblast and in some to the hypoblast." In other words, the development of certain parts of the alimentary canal may be so much delayed that they appear to arise from the epiblast.

This view is of special interest in considering the structures classed together as primitive streaks. As is well known, these structures are generally regarded as rudimentary parts of the blastopore (Balfour, Rauber). I would go further and suggest that it is an attempted development of that portion of the alimentary canal of the original ancestor which gave off the cœlomic pouches; that the portion which is not wanted in the development of simple larva of living animals is delayed, and consequently modified. I shall discuss this question at greater length in the second part of this paper.

I may conclude this part of my paper by describing briefly the ideal ancestor of the Cœlenterata and Triploblastic groups now under consideration, so far as the nervous system and mouth is concerned.

The Triploblastica and the Actinozoa are descended from a common two-layered bilateral ancestor which possessed an enlarged oral surface, an elongated mouth opening which by the adhesion of its middle portion was functionally divided into two openings, one at each end of the long axis of the mouth. The nervous system was generally distributed on the ectoderm all over the body, but was probably, as in living Actinozoa, especially concentrated on the oral surface. This type has persisted with certain modifications in Actinozoa, but in Peripatus and the other triploblastic forms under discussion the primitive mouth has completely divided, the body has elongated, and the nervous system has become especially aggregated in a ring (as in *Medusæ*) round the mouth and anus.

#### ON THE ORIGIN OF METAMERIC SEGMENTATION.

It has for some time been recognised that the body cavity or cœlom of the Triploblastica has been derived from diverticula of the archenteron. Such diverticula have been known for some time in the Echinodermata, *Sagitta*, *Brachiopoda*, *Balanoglossus*, *Amphioxus*.

The development of the body cavity in *Annelida*, *Arthropoda*, *Vertebrata*, and other cœlomate forms without diverticula has been supposed to be an embryonic abbreviation of this primitive process. I may quote the following passages from Balfour on this head.

“The formation of hollow outgrowths of the archenteron, the cavities of which give rise to the body cavity, can only be explained on the supposition that the body cavity of the types in which such outgrowths occur is derived from diverticula cut off from the alimentary tract. The lining epithelium of the diverticula, the peritoneal epithelium, is clearly part of the primitive hypoblast, and this part of the mesoblast is clearly hypoblastic in origin. . . . There can be but little doubt that the mode of origin of the mesoblast in many *Vertebrata*, as two solid plates split off from the hypoblast, in which a cavity is secondarily developed, is an abbreviation of the process observable in *Amphioxus*; but this process approaches

in some forms of Vertebrata to the ingrowth of the mesoblast from the lips of the blastopore.

“It is therefore highly probable that the paired ingrowths of the mesoblast from the lips of the blastopore may have been in the first instance derived from a pair of archenteric diverticula. This process of formation of the mesoblast is (as may be seen by reference to the summary (pp. 291, 292), the most frequent, including as it does the Chætopoda, the Mollusca, the Arthropoda,” &c. (‘Comp. Emb.’ vol. ii, pp. 293, 294).

It has been supposed until quite recently that only one pair of diverticula are developed (except in the Echinoderms and Balanoglossus). But Hatschek has shown that in *Amphioxus*, a very primitive and isolated animal, a series of diverticula are formed, each diverticulum giving rise to a mesoblastic somite, or, to put it in another way, that the lateral walls of the archenteron become folded before the region of the archenteron which they limit become separate from the central part of the archenteron. *Amphioxus* is the only segmented animal in which the body cavity is known to arise directly from archenteric pouches; development of the cœlom in other segmented animals being regarded as an abbreviation of a similar process. Now, however, that we know that the body cavity of *Amphioxus* is developed from a series of archenteric pouches, it seems to me that we are justified in concluding on similar grounds that the abbreviated development in other segmented forms is derived from a similar process.<sup>1</sup>

So that the difference between a segmented and an unsegmented animal consists in this, that in the former the archenteric walls become more folded than in the latter and give rise to a greater number of pouches, each of which becomes a mesoblastic somite. This is exactly the difference between a *Hydra* and a *Medusa*.

The similarity between the diploblastic *Amphioxus* embryo with a pouched gut (pouches giving rise to the mesoblastic somites) and an *Actinozoid* polyp or medusa suggests

<sup>1</sup> This has been already pointed out by Hubrecht; see Hubrecht, “On the Ancestral Form of the Chordata,” this Journal, 1883.



very forcibly the hypothesis that the mesoblastic somites of segmented animals are derived from a diploblastic Cœlenterate-like ancestor with folded gut walls, the folding having arisen as a result of the necessity for an increase in the extent of the vegetative surfaces in a rapidly enlarging animal.

I would venture, therefore, to suggest that Medusæ, Actinozoa and segmented animals are all derived from a common diploblastic ancestor, the Gastræa; that as this Gastræa increased in size it became necessary that some arrangement should arise by which a proper circulation of the nutritive matter to all parts of the body should be effected. For this purpose the gut wall became folded in such a way as to give rise to the radial and circular canals of Medusæ; to the mesenterial chambers (communicating peripherally by mesenterial stomata) of Actinozoa, and to the pouched diploblastic form from which segmented animals have arisen (I do not mean to assert that the segmented animals are the only animals which have arisen from a diploblastic animal with a pouched gut; vide below p. 60).

In a segmented animal the mesoblast is the first part of the body to show segmentation. The rest of the segmentation is moulded on the segmentation of the mesoblast. That is to say, the segmented organs, primitively at any rate, correspond in their segmentation with the somites. For each somite there is the nephridium, nerve ganglion, &c.

Supposing there is anything in the hypothesis I am putting forward, viz. that the somites of segmented animals are derived from gut pouches, which are homologous with the alimentary pouches of Cœlenterata, then it ought to be possible to explain on the same hypothesis the similar repetition of other organs.

In a segmented animal the following organs usually show the same repetition as the mesoblastic somites; the external appendages, the nephridia, the muscular system and the nervous system.

In Cœlenterata, both in Medusæ and Actinozoa—

(1) The tentacles correspond as a rule to the radial canals or to the mesenterial pouches;

(2) In *Medusæ* there are a number of pores leading from the circular canal to the exterior, placed on the oral side of the insertion of a radial tentacle, i. e. opposite a radial canal; in *Actinozoa* there are a number of openings in the body wall, putting the pouches in communication with the exterior (for function and possible origin of these pores vide below, p. 62).

(3) In *Medusæ* the circular striated muscles of the sub-umbrella are interrupted by the radial canals (*Hertwig*) and so broken up into a number of segments.

(4) In *Medusæ* there are sense organs which may be in connection with special nervous aggregations (*Acraspeda*) at the periphery of each radius.

In segmented animals—

(1) When segmented appendages are present (*Arthropoda*, *Polychæta*) they are simply processes of the body wall containing prolongations of the body cavity (*Peripatus*, embryonic *Arthropoda*).

(2) The *nephridia* are essentially pores leading from the body cavity to the exterior on the neural side at the base of the appendage.

(3) The muscular system is sometimes broken up into bands corresponding to the segments.

(4) The nervous system sometimes presents swellings, one for each somite.

I further venture to suggest that the greater number of the *Triploblastica* have arisen from *diploblastic* animals with a pouched gut; that in some of these, in consequence of the form taken by the body (elongation) and the consequent necessity for jointing and the persistence and greater development of the paired appendages, the body has become moulded, so to speak on this primitive gut pouching, which has therefore left its trace in the “segmentation”; that in unsegmented *Triploblastica*, in consequence of the action of causes of an opposite nature to those just mentioned, the pouches, after becoming separated from the gut, have become completely continuous with one another and left no traces. As a known instance of the latter process I may mention *Echiurus*

(Hatschek); in this animal (in the adult) most of the nephridia have been lost, the three pairs which persist (two pairs of brown tubes and anal vesicles) being enlarged and modified; the ganglionic cord is lost and there are no traces of the somites.

To sum up in a few words:—The Cœlenterata differ from segmented animals only in the fact that the alimentary or archenteric pouches (mesoblastic somites) and the alimentary canal do not become separate; and connected with this absence of a distinct cœlom is the low state of differentiation of such cœlomic structures as the excretory organs and the absence of a separate vascular system.

#### ON THE ORIGIN OF THE EXCRETORY ORGANS.

This part of my subject is so closely connected with the preceding that it is difficult to separate the two.

I have already referred to the Hertwigs' observations<sup>1</sup> on the marginal pores of Medusæ and the cinclides of Actinozoa.

Metschnikoff was, I believe, the first to observe these marginal pores in Medusæ, and he regarded them as excretory; in this view the Hertwigs concur.

There is, then, this common feature in the anatomy of the Medusæ and Actinozoa; they both possess peripheral pores, putting the alimentary pouches in communication with the exterior.

In the ACTINOZOA they seem to have an irregular distribution as tentacular pores and cinclides (vide Hertwig). In the Medusæ, however, they have a definite position, one pore for each radial canal.

It seems an obvious suggestion that in the less specialised ancestors of Medusæ and Actinozoa these pores were distributed more or less irregularly as in the Actinozoa: that their position was determined by the habits of life and form of the animal.

<sup>1</sup> Vide Hertwig 'Organismus der Medusen,' p. 39, and Hertwig, 'Die Actinien.'

It is worth while trying to picture how such pores may have arisen. In the supposed ancestor the two layers of the body wall were in more or less close apposition. The animal had no vascular system, and only one more or less differentiated opening, the primitive mouth. It would obviously be convenient that the excretory products should pass out as near as possible to the point where they were formed, or that there should be some arrangement of ducts by which they could be carried to the mouth opening. The latter arrangement does not appear ever to have been developed in the Coelenterata, while the former arrangement is present, if not in all, still in a great number of Medusæ and Actinozoa. My knowledge of the physiology of these low animals is not sufficient to enable me to offer any hypothesis of how the pores arose. But I may suggest that in the first instance the endoderm cells were of one kind only, whose function was to eat (in an amœboid manner) the food swept into the body cavity through the mouth opening, and to prepare soluble nutritive juices which passed to the ectoderm. The excretion of nitrogenous waste products must have been carried on by all the cells of the body, inasmuch as there is no circulatory system. The immediate undigestible remains or solid excreta from the endoderm cells would be cast into the alimentary cavity. Originally the latter must have been swept to the mouth and so got rid of. As the animal enlarged in size, and no well-developed canal apparatus appeared by which these solid waste products of the alimentary cavity would be directly carried to the mouth opening, some of the endoderm cells at the periphery of the animal became specially modified to eat these products, and pass them through or between the ectoderm cells to the exterior. So a close connection became established between the cells of the ectoderm and the endoderm, which eventually led to the establishment of a pore, the excretory pores. For an example of this kind of excretion through the ectoderm, I may refer to Eisig's<sup>1</sup> observations on

<sup>1</sup> "Die Segmentalorgane d. Capitelliden," 'Mitth. a. d. Zool. Stat. z. Neapel,' vol. i.



the Capitellidæ, in which the excretory organs end blindly against the ectoderm; their products, therefore, must pass to the exterior in some such way as I have suggested. If my suggestion be correct, it follows that the excretory organs were in their origin not specially organs for the excretion of nitrogenous waste products (each cell of the body being in close relation to the exterior did this itself) but for the riddance of the undigested and solid excretory products; and also that the excretory process was in its origin an intra-cellular process, i. e. temporary passages (amœba) were formed in the cells, through which the solid products passed to the exterior. This latter deduction is supported by the fact that in the higher animals the first formed excretory organs of the larva (Hatschek, Polygordius; Caldwell, Phoronis) have the form of delicate ducts attached to and opening through the ectoderm and ending in the body cavity, each in a simple cell; i. e. they are blind internally, and the excretory products in the body cavity must pass through the cell to get to the exterior.

Whatever view may be held as to the origin of the pores, the fact of their existence in the Diploblastica is undisputable.

At first irregularly arranged (a condition retained in Actinozoa, but more markedly in Sponges), they soon acquired a regular arrangement (Medusæ), and on the differentiation of the alimentary cavity into a digestive part (gut proper), and a circulatory and excretory part (cœlom), they remained in connection with the cœlom, which latter became again differentiated into parts purely excretory and connected with the pores (nephridia), and into the general vascular space for the circulation of the nutritive fluids passed into it from the endoderm cells.

Turning to the development of the excretory organs of the higher animals, we find that in the Vertebrata they arise as special parts<sup>1</sup> (not mere outgrowths) of the cœlom, and I have no doubt that this will be soon shown to be the case for the development of the Invertebrate excretory organs.

<sup>1</sup> Sedgwick, "Development of Kidney, &c.," 'Quart. Journ. of Mic. Sci.,' vol. xx, 1880.

Here, however, an apparent difficulty presents itself. In the Vertebrata the excretory organs (which probably were primitively segmental<sup>1</sup>) open not to the exterior direct, but into a longitudinal canal which opens behind into the alimentary canal; while in the Invertebrata each of them opens direct to the exterior.

As an explanation of this difficulty I suggest that in the Vertebrate ancestors the primitive alimentary cavity acquired a well arranged system of ducts, by which the peripheral excretory matters were carried to the part of the alimentary canal near the hind end of the primitive mouth (future anus), that in consequence the excretory pores were not wanted, and were either never developed or if developed lost. As confirmatory evidence I may refer (1) to the circular canal of the *Medusæ*, which might easily be conceived transformed into the Vertebrate segmental duct, the excretory organs themselves being developed from the outer part of the radial canals; (2) to the method of development of the anterior and least modified part of the Vertebrate excretory organ. In the osseous fishes and Amphibia the segmental or pronephric duct arises as a groove of the body cavity, and is therefore a direct product of the archenteric endoderm. In most Vertebrates the development of the segmental duct is much modified; but I pointed out some years ago that we can only get an intelligible explanation of the connection between the excretory tubules and the duct of the kidney by supposing that they originally developed in continuity, both as specialised parts of the body cavity, and that this method of development is repeated in the case of the anterior part of the kidney of Ichthyopsida, and in a more modified manner in the Amniota.

Turning to the Invertebrata, we find that the development is not direct from the *cœlom*, but from solid masses of cells<sup>2</sup>

<sup>1</sup> Elasmobranchs. For discussion of this question, vide Sedgwick, "Early Development of Wolffian Duct," 'Quart. Journ. of Mic. Sci.,' vol. xxi, 1881.

<sup>2</sup> Very various accounts are given of the origin of the Invertebrate excretory organs. I reserve a critical examination of these facts until I have worked out the development of the nephridia of *Peripatus*.

derived from its walls. This may reasonably be explained in the same way as I have attempted to explain in my paper quoted above, the development of the hinder part of the Amphibian kidney (modified larval development).

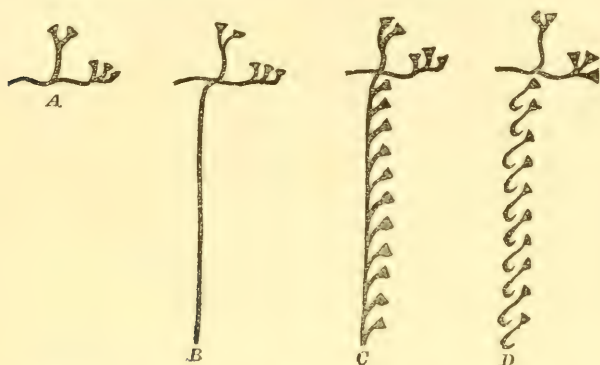


FIG. 1.—Diagram illustrating the development of the excretory system of *Polygordius* (from Balfour, after Hatschek).

The development of the excretory organs in *Polygordius* (woodcut, fig. 1) as described by Hatschek, is explicable on my hypothesis and so is confirmatory. The temporary longitudinal canal, which at first connects all the organs, is obviously a rudiment of the longitudinal duct found in the Vertebrata. The presence of this duct indicates that in the diploblastic ancestor of *Polygordius*, a system of canals was present in the cœlom together with the excretory pores.

#### ON THE ORIGIN OF TRACHEÆ AND GILL SLITS.

The view that tracheæ are derived from the cutaneous glands of a worm-like ancestor with a well developed middle layer is beset with so many physiological difficulties that I venture to suggest the following hypothesis, which agrees equally well with what we know of the development of tracheæ.

Tracheæ had their origin, like the organs so far discussed, in the diploblastic ancestor. In this ancestor they had the form

of simple ectodermic pits developed for the purpose of aerating those organs, whose position prevented their getting a sufficient supply of oxygen from the external medium or from the water circulating in the alimentary cavity. It must be remembered that there was no vascular system in this ancestor, and that therefore the living protoplasm of all parts of the body had to obtain its oxygen directly from the external medium. This method of aeration has persisted at the present day in certain *Medusæ* (sub-genital pits), in the *Tracheate Arthropoda*, and has left its trace in the *Vertebrata* in the canal of the central nervous system.

On this hypothesis the complicated distribution of tracheæ receives a physiologically satisfactory phylogenetic explanation.

The tracheæ were at first simple pits of ectoderm in a diploblastic animal, and they gradually became more complicated and branched as the other organs also became more complicated and folded.

The development of tracheæ fits in perfectly well with this view.

The tracheal respiration is then a primitive method of respiration, which has persisted in but few of the *Triploblastica*. It had its origin at a time before the vascular system was developed, and its essence consists in the fact that the living protoplasm takes its oxygen direct from the external medium. On this hypothesis the central canal of the central nervous system was a respiratory organ in a diploblastic *Vertebrate* ancestor without a well developed vascular system.

As soon as the vascular system became well developed, and the vascular fluid capable of carrying oxygen, the respiratory organs became localised. A special localisation of tracheæ is found in the pulmonary sacs of the *Scorpion*. In other animals external appendages have arisen. But in *Vertebrata*, *Balanoglossus*, and *Ascidians*, the circulation of water over the surface of the endoderm has been more developed. In the *Diploblastic* ancestor respiration was, as I have stated, partly effected by water circulating in the alimentary cavity. It entered by one end of the mouth and passed out



partly through the other end, and partly through the excretory pores of the alimentary pouches. Some of these alimentary pouches became, on the development of a vascular system, specialised as respiratory organs and retained their communication both with the exterior and with the alimentary cavity.

Thus gill slits are serially homologous with nephridia. This view of their origin is entirely supported by their development from pouches of the hypoblast of Vertebrate embryos, and by the fact that the kidney system in Vertebrata does not overlap them, but begins immediately behind them. A difficulty to this view lies in the fact that the coelom does overlap the gill slits; but I think this difficulty is not a serious one when we remember that the coelom being originally a vascular space had to extend in the region of gill slits as elsewhere, and that this extension might easily have proceeded either from the mesoblastic somite next behind the last gill pouch, or from a compression of the body in this region so that many somites (probably after separation from the archenteron) extended into the region of the gill slits.

#### SUMMARY.

The hypothesis suggested in the preceding pages are all based upon the gastræa theory, developed by Lankester and Hæckel. I take the gastræa as my starting point and do not inquire how the gastræa itself arose. I first (p. 48 to p. 57) by following the gastræa theory to its logical conclusion—and there seems recently to have been a disinclination on the part of some morphologists to do this—attempt to show that the gastræa mouth is not only homologous with the Cœlenterate mouth, but that the blastopore of the embryos of the Triploblastica is homologous with the gastræa mouth, and therefore homologous with the Cœlenterate mouth; and, finally, that if these necessary deductions from the gastræa theory are correct, and it should be noticed that the gastræa theory itself stands or falls with them—it necessarily follows (from the consideration of the *Peripatus* embryo) that the mouth and anus of the Triploblastica are derived from the gastræa mouth, i. e. Cœlenterate

mouth. I have pointed out that the blastopore in becoming the larval mouth must have become highly specialised and unable in most cases to repeat its ancestral history in the larval development, and that the behaviour of the blastopore becomes much more intelligible, though, I admit, not entirely so.<sup>1</sup> The remainder of my hypotheses are simply following the lines of the recent speculations on the origin of the nervous and muscular tissue. My speculations, like these, are based (1) on facts of Cœlenterate anatomy which have been mainly brought to light by the magnificent work of the Hertwigs. (2) On facts of embryonic development which have been for the most part long known, but have recently been added to in an important manner by Hatschek's work on *Amphioxus* and Balfour's discovery of the embryo of *Peripatus capensis*. The object of my speculation has been to extend Balfour's theory of the Triploblastic nervous system to the remaining systems of organs; in other words, I have attempted to show that the majority of the Triploblastica (I confine myself to the Annelida, Arthropoda Mollusca, Vertebrata and certain small groups, e.g. *Balanoglossus*, *Sagitta*, *Brachiopoda*) are built upon a common plan; and that that plan is revealed by a careful examination of the anatomy of Cœlenterata: that all the most important organ systems of these Triploblastica are found in a rudimentary condition in the Cœlenterata; and that all the Triploblastica referred to must be traced back to a common diploblastic ancestor common to them and the Cœlenterata.

<sup>1</sup> I shall return to a consideration of the behaviour of the blastopore in the second part of this paper.

## PART II.

## APPLICATION OF THE ABOVE HYPOTHESES TO THE VERTEBRATA, ANNELIDA, ARTHROPODA, MOLLUSCA, AND CERTAIN SMALLER GROUPS.

Fig. 7 represents a diagram of the ideal ancestor of all the above-mentioned Triploblastica. It closely resembles the common ancestor of the Cœlenterata but may be supposed a little more advanced in specialisation. For instance, the peripheral excretory pores (*o*) have a regular arrangement. This animal is supposed to have a bilateral symmetry shown in the gut pouches and in the excretory pores. It is supposed to have an elongated mouth partly differentiated into two parts, and the nervous system is generally diffused over the oral surface (which will henceforth be called the neural surface) with a tendency to specialisation into a narrow tract.

This ideal ancestor soon gave rise to two stocks, the first differences between which may be supposed to depend on the shape of the body.

In the one stock the mouth and anus (which soon became separated) remain on the neural surface, a præoral lobe was developed on the abneural surface of the body (fig. 12); this præoral lobe being carried first in movement became specially sensitive and the nervous system largely developed.

This stock is the Invertebrate stock. The præoral part of the nervous ring in consequence of the shape the body has taken becomes enlarged and sense organs largely developed in connection with it. The hinder præanal parts of the nervous ring have more or less approximated to each other, and are connected by commissures and become swollen at intervals where many nerves pass out to the locomotive organs (appendages). The postanal part of the ring becomes weak and often disappears, never having more than a commissural function (absence of nerve-cells in postanal connection of lateral nerve trunks of *Peripatus*, vide Balfour on *Peripatus capensis*).

With regard to the endodermal organs the alimentary pouches have lost not only their connection with the alimentary cavity and now constitute mesoblastic somites (fig. 8), but have also lost their peripheral connection with each other. The excretory pores persist and the part of the somites near the pore becomes developed into the nephridia.

In the other stock the body assumed a different shape, in consequence of which the mouth and anus became terminal (vide fig. 13, ideal). A projection overhanging the mouth then appeared on the neural surface and gave rise to a neural præoral lobe (fig. 14.) The præoral and postanal part ( $N^1$  and  $N^2$ ) of the nervous ring soon became inconspicuous and vanished. (It must be remembered that the nervous system of this stage of evolution was little, if at all, more developed than that of living Actinozoa.) This is the stock of the **Vertebrata** and **Balanoglossus**. The part of the primitive ring immediately behind the mouth is the most important in this stock; it is placed at the anterior end of the body, and therefore enlarges and develops sense organs. (Fig. 14.)

With regard to the endodermal organs the pouches have become differentiated into two kinds:

(1) Anteriorly a certain number retain their communication with the exterior and with the gut. (Fig. 10.)

(2) The majority, however, lose their connection with the gut and with the exterior, but remain connected by the peripheral canal, which behind retains (by means of a pouch?) its communication with the gut.

(3) A posterior pouch loses its connection with the gut and with the longitudinal canal, and gives rise to an abdominal pore.

The first group of pouches become the gill slits, the second become the cœlom, while part of each of them become differentiated into nephridia which opens into the longitudinal canal (pronephric or segmental duct). The last pair of pouches gives rise to a part of the cœlom and retains its connection with the exterior as an abdominal pore.

The further evolution of the **Invertebrate Stock**.—Paired



processes of the body wall (fig. 10), into which the cavities of the somites were continued are present (generally homologous with tentacles of Cœlenterata which correspond with the mesenterial chambers or radial canals). These become specially locomotive, and consequently muscular; hence the swellings (ganglia) on the nerve cords, each swelling corresponding to appendages, i. e. to a somite.

The septa between the pouches have more or less broken down, so that the cœlomic spaces become connected; the dorsal or ventral mesenteries, both or one of them, likewise break down.

Sometimes the appendages vanish (Gephyrea, Mollusca), the ganglionic swellings then disappear, and the only trace in the adult of the embryonic segmentation is seen in the nephridia. Many of these must, however, have vanished (according to Hatschek's account of development of Echiurus), and two or three or four pairs have become enlarged and alone persisted. It is interesting to notice the differentiation of the persisting nephridia in the Gephyrea into the brown tubes, which act as excretory organs and generative ducts, and the anal vesicles. This differentiation of the nephridia of different parts of the body is carried, as we shall see, much higher in the Vertebrata. In the Mollusca the disappearance of the somites has gone even further than in the Gephyrea, and the cœlom has become much modified. In Nautilus, however, a trace of the original segmentation persists in the nephridia and vascular system.

The development of *Sagitta* indicates that it is derived from an ancestor with three pairs of pouches, two of which retain their external pores (generative orifices). The Brachiopoda I at present leave out of special consideration.

Thus, the number of pouches (segments) in the Triploblastica varies in different cases, just as do the alimentary diverticula of the Actinozoa.

The further evolution of the Vertebrate Stock.—The central nervous system which is almost entirely derived from that part

of the primitive ring intervening between the mouth and anus, unites more or less completely across the middle line.

It and the superficial epiblast with which it is in connection become grooved; the groove becomes deepened and converted into a canal open close to the mouth in front and close to the anus behind (fig. 15).

The function<sup>1</sup> of this canal at this stage (the siphon stage) I have elsewhere discussed and ventured to suggest that it was in the main respiratory. (For the embryological counterpart of the siphon stage, see below, p. 75.)

It is important to notice that the nervous system of the Vertebrata becomes removed from the surface in quite a different way to that which obtains in the Invertebrata. In the latter it becomes removed from the surface by the ingrowth of mesoblastic tissue between it and the superficial layer in connection with which it arose. In the former, on the other hand, it never separates from the superficial epiblast from which it arises. The latter is involuted with the nervous mass and persists through life as the lining of the canal of the Vertebrate nervous system. This fact is of great importance in speculating on the origin of the Vertebrata, for it shows that the Vertebrate stock is a very primitive one, and must have separated from the Invertebrate stock before the nervous system of the latter separated from the epidermis.<sup>2</sup>

It will be observed that in consequence of the development of the præoral lobe (fig. 15 not marked enough), the mouth has become placed on the other side of the body, i. e. on the abneural side, and the neural canal has to bend towards this surface (the future ventral surface) in order to open into the mouth.

The water which was attracted by the ciliary movement

<sup>1</sup> For a discussion of the function of the canal at this stage, vide Sedgewick, "On the Original Function of the Canal of the Central Nervous System of Vertebrata," 'Proceedings of the Cambridge Philosophical Soc.,' vol. iv.

<sup>2</sup> This fact also holds for the cerebral ganglia of *Peripatus*; the invaginations of ectoderm become constricted off, and their cavities persist throughout life in the ventral protuberances of the brain.

divided at the anterior opening (fig. 15) into two streams, one of which passed through the mouth into the alimentary canal, while the other passed through the neural canal.

There was probably an olfactory sense organ developed from the epiblast close to the front end of the neural canal over which this water rushed.

The anterior convex wall of the neural canal now becomes bulged out forwards, and gives rise to a large anterior lobe, whose cavity opens behind into the neural canal, close to its opening into the mouth (fig. 16). This anterior lobe carries with it the olfactory epithelium, which, however, remains in connection with the mouth by grooves or canals. It becomes bi-lobed and transformed into the cerebral hemispheres of living Vertebrates.

The neural canal now closes both in front and behind, and assumes some other function than that of respiration. Behind the closing leaves no trace, while in front remains of the connection are seen at the present day in the infundibulum, and in the pituitary body.

It will be evident from the above hypothetical account of the origin of the Vertebrata, that I believe that the mouth and anus of the Vertebrata are homologous with the corresponding structures in the Invertebrate segmented animals. I have stated above, that I suppose that the blastopore of the Vertebrata is a specialised larval structure derived from the primitive mouth of a two-layered ancestor. It will be obvious also, that, according to my view, the position of this primitive mouth coincided with the middle line of the dorsal surface of the Vertebrate embryo, and that supposing it persisted in its primitive form in the embryo until the adult mouth and anus were formed, it would appear as a slit extending from the mouth anteriorly and ventrally round the front end of the head, along the whole surface of the medullary groove to the primitive streak round the hind end to the ventrally placed anus.

In the first part of this essay which deals with the blastopore, I have attempted to show that the mouth and anus of

segmented Triploblastica are in all cases derived from a primitive single mouth; that this primitive mouth is represented in the embryo by the blastopore which should, if the phylogenetic development were repeated, give rise directly to the mouth and anus. I explained the fact that the blastopore so rarely does give rise to the mouth and anus by supposing that it became specialised as a larval<sup>1</sup> structure. My view is that in those animals in which it does not give rise to the mouth and anus, it functioned as the larval mouth while the animal was developing, and persisted until parts of the embryo were developed between it and the position of the mouth and anus of the adult, which parts had arisen in the phylogenetic history in the adult after the primitive mouth had completely divided into the mouth and anus. These parts never had been traversed by the original slit-like mouth, because they had appeared at a stage in evolution subsequent to the stage in which the mouth and anus were one. It cannot therefore be a matter of surprise if the blastopore does not elongate and bisect these later structures, which never had in the history of the animal been perforated by the blastopore. It is very difficult for me to express my meaning in clear language, and I am driven to take an instance to illustrate it. According to my view the cerebral hemispheres have appeared at a stage in the evolution of the Vertebrata long after the primitive mouth has become separated into the mouth and anus. The blastopore (primitive mouth), however, which has in some ancestral Vertebrate functioned for a considerable time in the larva as the only opening into the alimentary canal, persists and does not elongate to give rise to the mouth and anus which are not formed until after the cerebral hemispheres have appeared. It is now no longer possible, nor would it be advantageous if it could, that the specialised blastopore should elongate and give rise to the mouth and anus, the middle part closing up. The cerebral hemispheres have appeared, and they have never in the phylo-

<sup>1</sup> The larval stage, for which the mouth was specialised, has in the Vertebrata, as in many other animals, vanished; it has probably been included in the embryonic period, and rapidly hurried over.



genetic history been traversed by this slit. Consequently the only course open is that the mouth should be formed as a secondary perforation entirely independent of the blastopore.

From the nature of the case it is exceedingly difficult to bring forward any direct proofs derived from embryology in favour of this view. But I think it can be shown that there is reason to believe that the mouth and anus of the Vertebrata are placed in the line of the original blastopore. Amphioxus, so far as I understand its development, offers no support to my view, but the case is different with the Ascidians and the higher Vertebrata.

Weldon<sup>1</sup> has shown conclusively that the anus is formed within the area of the primitive streak, though after the disappearance of the latter structure. It is on all hands admitted that the primitive streak is a part of the original blastopore. I need, therefore, say nothing with regard to the anus.

The mouth, however, is a great difficulty. Dr. Dohrn has attempted to show that it is derived from a pair of gill slits. Now, without considering the embryological facts opposed to his view, which have been so ably pointed out by Balfour, I venture to suggest that it is exceedingly improbable that an animal should lose its mouth and develop a new one. It is surely, on a priori grounds, far more likely that it would change gradually the position of its mouth than that it should lose it and go through the labour of acquiring a new one, though that new one is supposed to be derived from pre-existing structures.

Turning to the actual development, I may mention here two facts which appear to me of importance.

(1) In Ascidians, Kowalewsky<sup>2</sup> has shown that the mouth at a certain stage is dorsal (neural), and that the neural canal opens into it (woodcut, fig. 2, V). The neural canal, also, at a slightly earlier, if not contemporaneous stage, opened behind into the gut. We thus find the hypothetical siphon stage of the evolution of the neural canal actually repeated in the

<sup>1</sup> 'Quart. Journ. of Mic. Sci.,' 1883.

<sup>2</sup> Kowalewsky, 'Arch. f. Mic. Anatomie,' vol. vii, 1871.

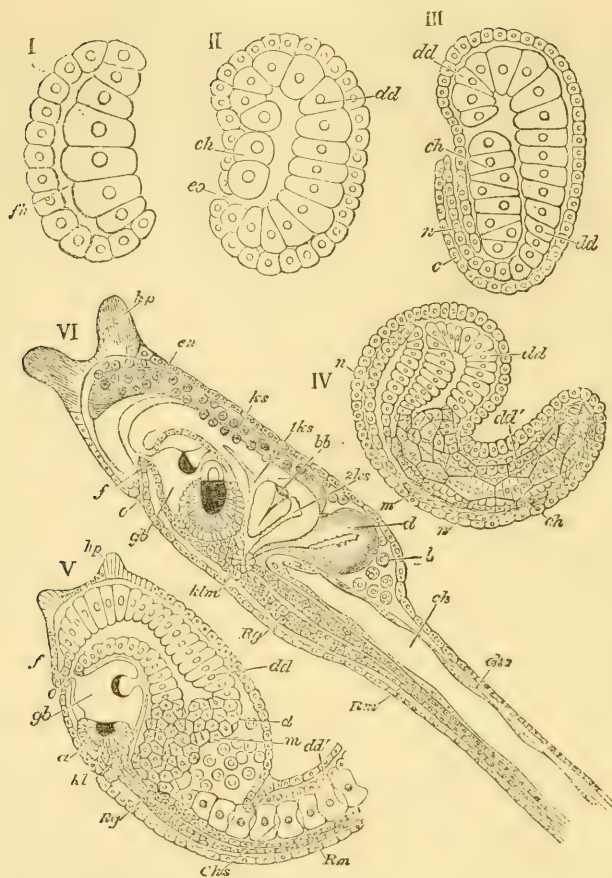


FIG. 2.—Various stages in the development of *Phallusia mammillata* (from Huxley; after Kowalewsky). I. Commencing gastrula. *fh*. Segmentation cavity. II. Late gastrula stage. *eo*. Blastopore. *ch*. Notochord. *dd*. Hypoblast. III. More advanced embryo. *n*. Neural tube. *e*. Epiblast. IV. Formation of neural tube completed. *dd'*. Hypoblast in tail. *m*. Muscles. V. Larva just hatched, the end of the tail is not represented. *a*. Eye. *gb*. Dilated extremity of neural tube, with otolith projecting into it. *Rg*. Anterior swelling of spinal division of neural tube. *f*. Anterior pore of neural tube. *Rm*. Posterior part of neural tube. *o*. Mouth. *chs*. Notochord. *kl*. Atrial invagination. *dd*. Branchial region of alimentary tract. *d*. Commencement of oesophagus and stomach. *dd'*. Hypoblast in tail. *m*. Muscles. *hp*. Papilla for attachment. VI. Body and anterior part of a two days' larva.

development of a living form. Salensky<sup>1</sup> long ago pointed this out. With a slight change in the shape of the anterior end of the body of the Ascidian larva in Kowalewsky's figure, the mouth would be removed from what we call the dorsal (neural) to what we call the ventral (abneural) surface. This would involve a flexure of the anterior end of the neural canal, and, I think, gives a clue to the phylogenetic meaning of the cranial flexure. The closure of the anterior pore of the neural canal is effected in such a way that it leaves a trace on the one hand as the infundibulum; on the other as the pituitary body. This homology has been often suggested. The persistence of the lower part of this pore, and its development from the epiblast of the buccal cavity, may be explained by supposing that the buccal end of the pore was glandular before the closure of the neural canal was effected. When this closure was effected, the buccal part remained in connection with the mouth as an excretory organ, a state of things persisting, according to Salensky, in Ascidians. It then acquired the new functions which it has at present, lost in the adult its connection with the mouth, and is known to us as the pituitary body. Meanwhile, some of the endoderm cells of the dorsal wall of the alimentary canal have become specially modified and separated from the rest as the notochord.

(2) In the Vertebrata the anterior end of the notochord is bent round, and becomes connected with the pituitary body at its extreme front end.<sup>2</sup> This condition of the anterior end of the notochord may be seen in the embryo before the pituitary involution is cut off from the ectoderm of the developing mouth—that is to say, the relation of the anterior end of the notochord to the ectoderm is similar to that of the hind end;

<sup>1</sup> Salensky, 'Zeitschrift f. Wiss. Zoologie,' vol. xxvii, p. 212; and 'Morphol. Jahrbuch,' vol. iii, p. 600.

<sup>2</sup> The relation of the anterior end of the notochord to the pituitary body is somewhat complicated. For the knowledge of this fact I am indebted to Mr. Heape, who is at present engaged in investigating this very point. He informs me that the existence of the connection was known to the older embryologists (W. Müller).

behind it is closely connected with the front wall of the neurenteric canal; in front it is closely connected with the ectoderm of the developing buccal cavity.

At a still earlier stage, before the cranial flexure has appeared, the front end of the notochord is swollen, and runs into and is continuous with the front end of the medullary plate. This state of things I have myself observed at a stage before the medullary plate has begun to fold. Now, my view is that this connection of the notochord marks the site of the future mouth; that the site of the mouth—at first as in *Ascidians*—perforates the medullary plate, and is on the dorsal surface; that soon, however, this site bends round on to the ventral surface, and is eventually invaginated to form the buccal cavity and pituitary body. This hypothesis can easily be tested in the chick with the new Caldwell automatic microtome, but I regret that I have not hitherto found time to do so.

(Professor Hubrecht in his ingenious paper already quoted (this Journal, 1883), has instituted a comparison between the pituitary body and notochord of Vertebrates and the proboscis and proboscis sheath of *Nemertines*.)

The cerebral hemispheres appear relatively late in front of the notochord, and this fact fits in very well with the account of their origin which I have suggested. On this view *Amphioxus* has separated from the vertebrate stock before the appearances of the cerebral hemispheres.

The modification of the alimentary pouches, and the longitudinal canal connecting them, I have already alluded to. It only remains for me to point out that the cavities of the mesoblastic somites soon come to communicate ventrally both with each other and those of the opposite side; that the dorsal mesentery for the most part only persists, though the ventral mesentery remain in the region of the heart, liver, and behind in the region of the hind part of the body; that the nephridia become modified into groups, each with a special importance; the pronephros, or larval organ, is the first formed part of the kidney and atrophies in the adult; the hinder part differentiates



into meso- and meta-nephros; the meso-nephros becomes connected with the male generative organs, and loses its excretory function, while the metanephros persists as the functional kidney. I have, however, fully discussed the evolution of the Vertebrate excretory system in my papers already quoted on their development, and need not refer further to it here, except to point out that there is every reason to believe that the nephridia were originally segmental, one for each somite, that this segmental arrangement is, with the specialisation of the kidney, soon lost as it is in other organs.

#### ON THE STRUCTURES KNOWN AS PRIMITIVE STREAKS.

I may conclude this paper by a short review of these structures.

(1) They are always connected with the formation of the mesoblast.

(2) They are never, so far as I know, found in free larvæ. They are confined to the embryonic phase of development, and are only found in animals which undergo a considerable part of their development in the egg; in other words, only in eggs well-stocked with food yolk, or in eggs which have lost the food yolk. On the other hand, a primitive streak is not universally present in such cases, e.g. Cephalopoda, Elasmobranchii, Amphibia, Crustacea.

(3) They are always median and unpaired in their origin, but may in later development become grooved and present traces of a bilateral structure.

(4) They are always caused by rapid proliferation of cells, apparently from the epiblast.

(5) Their position seems to vary in different animals.

In Vertebrata, when present, the primitive streak is placed mainly behind the blastopore (according to Strahl<sup>1</sup> not entirely so in *Lacerta*, but this is not quite clear from his figures).

In *Peripatus* it is placed behind the blastopore, and, when the blastopore has divided, behind the hinder division (fig. 4).

<sup>1</sup> 'Arch. f. Anat. u. Phys.,' 1882.

In other Arthropoda in which a primitive streak is present, its position with regard to the blastopore cannot be determined; because the blastopore is not present in those cases in which there is a primitive streak.

With regard to the two first cases the blastopore of the Vertebrata closes, and the anus is subsequently (very late) formed within the area of the primitive streak.

In *Peripatus*, however, the hinder division of the blastopore does not close but travels slowly back over the area occupied by the primitive streak to its position at the hind end of the body.

I may here mention a fact which I observed last summer in the newt (*Triton cristatus*). In this animal the blastopore appears not to close but to persist as the anus. This statement is based on surface views of a large number of embryos from the stage when the egg is round until hatching. In all these stages I never saw an embryo without an opening at the hind end of its body. I very much regret that I have not had time to confirm this observation by means of sections.

If true it is most interesting as being the only known case in which the blastopore of Vertebrata actually persists as the anus.

In the case of larvæ which leave the egg at an early stage of development, no primitive streak is developed, but the mesoblast partly grows in from the lips of the blastopore, and partly arises as mesenchyme.

In *Amphioxus* fourteen pairs of somites are derived as hypoblastic pouches, the remainder are formed from hypoblastic tissue, the exact behaviour of which is not explained by Hatschek.

In those Vertebrata with primitive streak, the anterior somites may be regarded as arising from hypoblastic mesoblast; but the greater part are formed from primitive streak mesoblast.

In *Peripatus*, the mesoblast arises behind the blastopore from the primitive streak, and grows forward as two bands, exactly as in worms; but it arises from a primitive streak.

I do not think any really satisfactory explanation can be offered at present of these facts. I venture, however, to suggest the following as an attempt at an explanation.

In many living Triploblastica the embryo leaves the egg at a very early stage as a larva; at a stage in which it is little more than a gastrula. Inasmuch as the parent of this ancestor has differentiated nephridia and muscles, &c., it is easily conceivable that the larva should precociously acquire as much of these organs as it requires. Hence mesenchyme. This larva is a small animal, and does not require a pouched gut; its hypoblast becomes specialised for digestion; now it would obviously hamper these exceedingly active larvæ if the gut repeated the phylogeny; at any rate, it is easily conceivable that it would be more advantageous if it were possible that the digestive cells should not have to undergo active developmental changes. Hence the mesoblast has to be formed in another way. The methods in which it is formed are, as is well known, various; it nearly always, however, originates at the lips of the blastopore, as the result of the proliferation of a cell, or cells, which do nothing else but divide and give origin to the mesoblastic bands. This, as I have suggested above, may be looked upon as a modified development of that of the ancestral archenteron, which became pouched, and gave rise to somites (secondary invagination).

In those animals in which this larval phase has become merged in the embryonic development, this process is continued; but the area from which the major part of the mesoblast arises, i. e. from which the secondary invagination takes place, is larger. This may obviously be explained as being due to the fact that, the development being protected, it is not important that the amount of growing tissue present at any given moment should be as small as possible, in order not to hamper the larva.

On this view *Amphioxus* presents a most surprisingly primitive development, so far as its somites are concerned.

I need hardly point out that the prevailing order of develop-

ment, from before backwards, is just what would, a priori, be expected. The larva, being a free swimming animal, requires sense organs; it therefore develops its anterior part first and the organs belonging to this region of the body.

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On Certain Abnormalities in the Common Frog  
(*Rana temporaria*).

1. The Occurrence of an Ovotestis.
2. Abnormalities of the Vertebral Column.

By

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With Plate IV.

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1. ON the occurrence of an Ovotestis:

While much has been written, especially by human anatomists concerning hermaphroditism, and numerous cases have been described as abnormalities in which the generative organs in the one sex, from imperfect or abnormal development, approach the condition of those in the other sex, little is known concerning the existence of true hermaphrodite glands in the Vertebrata.

Two or three cases have been described of the existence of both ovary and testis in man. In one described by Heppner, of St. Petersburg,<sup>1</sup> small ova in Graafian follicles were found in one pair of glands and spermatic cells and seminal tubules in the other.

There are, however, certain fishes which normally possess hermaphrodite glands<sup>2</sup> (*Serranus scriba*, *S. cabrilla*, *S. hepatus*, and *Chrysophrys aurata*).

<sup>1</sup> "Ueber d. wahren Hermaphroditismus beim Menschen," in 'Müller's Arch.,' 1870, p. 676.

<sup>2</sup> See Dufossé, "De l'Hermaphroditisme chez certains vertébrés," in 'Ann. Sci. Nat.,' Sér. 4, "Zool.," tom. v, 1856. See also a memoir in Polish ex-

In view of our present knowledge of the undifferentiated character of the primitive generative cells, such cases of hermaphrodite glands, whether occurring as the rule in a species or as an abnormality in an individual, acquire a special interest.

The undifferentiated primitive ova being potentially either ova or spermatozoa, it is interesting to find that in any one gland some have become ova and some spermatozoa; it is in fact what we might expect to occur from time to time.

Among the Invertebrata which exhibit the hermaphrodite condition we have various stages differing in the completeness with which the hermaphroditism is expressed. We may distinguish between forms where the glands are completely separate, those in which male and female portions remain connected as regions of one and the same gland, and lastly, forms in which the male and female elements are more completely mixed, where ova and spermatozoa develop from contiguous cells of a single follicle. In the ovotestis of the frog here recorded certain follicles of the gland have become ovarian, others testicular, ova and spermatozoa both being well developed.

The specimen presented a well-developed ovary upon the right side, while upon the left was the organ in question. Both oviducts were normally developed. Unfortunately the specimen was cut about before it came into my hands, and I was unable to observe any vasa efferentia connected with the testicular portion of the ovotestis. Both ureters presented the normal female arrangement.

The ovotestis (Pl. IV, figs. 1 and 2) was completely ovarian in its posterior half. There was no special line of separation between ovarian and testicular portions, but as may be seen from the figures, there was a complete intermixing of the two elements. The testicular portion was rounded, and tended to assume the shape of a normal testis, it contained motile spermatozoa. Although the specimen was in a bad state of pretracted in 'Schwalbe and Hofmann's Jahresbericht,' vol. v, 1878, p. 345. Also Brock, "Beiträge zur Anatomie u. Histologie der Geschlechtsorgane der Knochenfische," in 'Morph. Jahrbuch,' Bd. iv, 1878.

servation, I cut sections of a portion of the gland and found that neighbouring follicles had developed some ova, others spermatozoa, both in a normal manner.

In this connection a complete study of certain organs which occur in toads would doubtless yield most interesting results. These organs were first found by Jacobson<sup>1</sup> in *Bufo cinereus*, in males, and he regarded them as rudimentary ovaries.

Von Wittich<sup>2</sup> describes and figures these organs, and upholds the view that they are rudimentary ovaries, and afford evidence of the primitively hermaphrodite nature of the generative glands in Amphibia.

They are also described by Bidder,<sup>3</sup> who regards them as accessory male glands serving to carry on the first stages of the development of the spermatozoa.

Spengel<sup>4</sup> has more recently described these organs, and states that in addition to being found in males, where they persist, such organs exist at the anterior extremity of the ovary in young females in all Bufones, rendering it—he argues—in the last degree unlikely that the organ is a rudimentary ovary. Spengel cites, in confirmation of his view, a specimen of *B. cinereus* which came under his notice, where on each side was a well-developed testis, and above this a true ovary, while between the ovary and the corpus adiposum was placed this organ which he terms “Bidder’s organ.” Spengel also mentions an abnormal specimen of *Pelobates fuscus*, which resembles very closely my specimen of *Rana temporaria*. On the one side was a normal testis, while on the other the anterior half of the gland was a testis and

<sup>1</sup> ‘Danske Videnskabernes Selskabs Naturvidenskabelige og Mathematisk Afhandlingar,’ 1828, p. xlii.

<sup>2</sup> ‘Beiträge zur Morphologischen und Histologischen Entwicklung der Harn und Geschlechtswerkzeuge der nackten Amphibien,’ ‘Z. f. w. Zool,’ Bd. iv, p. 125.

<sup>3</sup> ‘Vergl. Anat. u. Hist. Untersuchungen über die Männlichen Geschlechts- und Harnwerkzeuge der nackten Amphibien,’ Dorpat, 1846, p. 27, &c.

<sup>4</sup> ‘Das Urogenital-system der Amphibien,’ ‘Art. aus dem. Zool. Zoot. Inst. in Würzburg,’ Bd. iii, 1876-77.

the posterior half an ovary. In this case no trace of Muller's duct (oviduct) was present on either side.

Spengel comes to the conclusion that, as stated by Bidder, this organ is not a "rudimentary ovary," but is possibly connected with the early stages of the spermatozoa. How then are we to account for its appearance in the female?

In order to offer a definite opinion upon the subject it would be necessary to trace the earliest stages of development of this organ and also to ascertain if it undergoes any periodic change in either sex. This, up to the present, I have been unable to find leisure to do.

I have, however, obtained sections of the organ in connection with the testis in an adult male, *Bufo cinereus*, and figured it (fig. 4) for comparison with the section of the ovotestis of my specimen of *Rana temporaria* (Fig. 3). It will be seen from these figures that the two glands are similar, and that it is probable that Jacobsen and Von Wittich were right in regarding this organ in the toads as a rudimentary ovary in the male. That it occurs in the female does not, I think, necessarily militate against this view. An account of its development here would be most interesting.

## 2. Abnormalities of the Vertebral Column:

Two cases of abnormal vertebral column have come under my notice, both showing the same tendency, viz. an increase in number of vertebræ.

Case A, Pl. IV, figs. 6—8.—The atlas possesses transverse processes which run outwards and very slightly forwards, the centrum is normally developed, but the neural arch is slightly asymmetrical. Fig. 6 shows the dorsal aspect, fig. 7 the ventral.

In the axis the transverse processes are directed forwards instead of backwards, and that of the left side presents an indication of bifurcation at the extremity.

The third vertebra possesses two pairs of transverse processes which are joined together for two thirds of their length.

The fourth vertebra presents a transverse process which is bifurcated at the extremity.



The remaining vertebræ, although slightly asymmetrical, present no remarkable peculiarity, except that the neural arch of the ninth vertebra is feebly developed.

Case B., Pl. IV, figs. 9—12.—The axis and third vertebræ both possess tubercles upon the transverse processes, probably an indication of a tendency to bifurcation as seen in vertebræ two and four of Case A.

The ninth vertebra is abnormal, in that on the left side it possesses a rudimentary, and on the right side a well developed posterior zygapophysis, the latter overlapping the anterior zygapophysis of a tenth vertebra.

The general shape of the ninth vertebra and its large transverse processes show that it is homologous with the ninth vertebra of the normal vertebral column; the vertebra behind it, the tenth in the series, is therefore a portion of the urostyle which has become segmented.

This tenth vertebra has an imperfect centrum and only a single anterior zygapophysis articulating with the zygapophysis of the ninth vertebra. There are short pointed transverse processes which are directed backwards. The neural arch is complete but asymmetrical, and the neural canal is of considerable size (fig. 11).

The centrum of the ninth vertebra has posteriorly two concavities not two convexities as in the normal Frog, while that of the tenth vertebra presents two convex articular surfaces fitting into the two concavities in the urostyle.

The Anura may present one or two pairs of transverse processes placed one behind the other at the proximal end of the urostyle (*Discoglossus*). And this additional vertebra occurring as an abnormality in the common Frog is interesting as showing the potentially segmented character of the urostyle, and its homology with the caudal vertebræ of Urodelous Amphibia.

The transverse processes on the atlas in Case A, and the bifurcated nature of so many of the transverse processes seem to indicate a tendency to increase the number of vertebræ, and

to revert to the more primitive Amphibian condition, found in the Urodela.

I regret that the specimens came into my hands as skeletons so that no knowledge of the arrangement of the spinal nerves could be obtained.

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## Researches on the Intracellular Digestion of Invertebrates.

By

**Dr. Elias Metschnikoff.<sup>1</sup>**

I HAVE long been of opinion that many questions connected with the genealogy of the Metazoa are not to be solved by the methods of purely morphological embryology. Morphology certainly considers as of primary importance the structure of organs which have either lost their function by retrogression, or which, being not yet fully developed, have not acquired their full functional activity ; but in the determination of the phylogenetic importance of such organs, a knowledge of their physiological history is often indispensable. The embryonic history of an animal or an organ shows us a series of phenomena, often extremely complicated, among which mere embryology cannot in many cases choose out those which are primitive from those of secondary value. Every student of comparative embryology knows how hard it is to determine in any given case the genealogical meaning of a certain phenomenon, and how often the standard used is purely subjective. These difficulties are increased by the fact that the primitive Metazoa have all disappeared, so that the gap between the Metazoa of to-day and the Protozoa is wide indeed.

From what has been said, it is evident that, in attempting to discuss the evolution of the alimentary canal, one of the oldest and most widely distributed of all the organs of the Metazoa, one must collect not only embryological evidence as to the mode of formation of the endoderm, but also physiological evidence as to its function.

<sup>1</sup> Translated from the "Arbeiten a. d. Zoolog. Instit. Wien.," 1883.

When it became known that all the lower Metazoa, such as Sponges, Cœlenterates, and Turbellarians, possessed an intracellular digestion, the conclusion was obvious that this mode of nourishment was one of the few characters in the organisation of the Metazoa, which had been directly transmitted to them from the Protozoa, and so constituted a link, however small, between the two groups. Now, since the colonial Monads—organisms which most closely resemble the lowest known Metazoa, their embryos and larvæ—show no kind of division of labour, no separation into nutritive and locomotive individuals, the question arises whether the lowest Metazoa have not retained the power of using any or all the cells of their body for the purpose of ingesting food.

In order to answer this question I undertook, in the course of last year, and especially during a six months' residence in Messina, a series of investigations, the chief results of which will be described in what follows. That portion of my work which relates to intracellular digestion in the endoderm itself, I reserve for a future paper.

#### I.—INTRACELLULAR INGESTION BY ECTODERM CELLS.

Sponges, in which, seeing that they are the lowest Metazoa at present known, one would most especially look for some kind of ectodermal ingestion of food, are not suited for detailed observation; because the ectoderm in the living creature is either invisible or else can be seen only imperfectly. When Krukenberg speaks of a digestion of albuminoid bodies by the "ectodermal covering" of many sponges, it is not evident whether he really means the delicate sheet of flat epithelial cells which constitutes the true epidermis of these animals. In any case, it is not possible, by such experiments as his, to determine the part played by wandering mesoderm cells immediately below the thin ectoderm. Von Lendenfeld, in a recently published memoir on Australian Aplysiniæ,<sup>1</sup>

<sup>1</sup> "Ueber Cœlenteraten der Südsee," ii, 'Zeitschr. f. wiss. Zool.,' Bd. xxxviii, 2, 1883, p. 253.



speaks much more precisely. He states that in these sponges the ectoderm is capable of taking up foreign particles, but not of digesting them, the ingested matter being simply passed on to the wandering cells of the mesoderm. However plausible this view may seem, it appears, from Von Lendenfeld's statements, to need confirmation.

My own researches, conducted on *Ascetta primordialis* and *Halisarca lobularis*, two sponges with strongly developed ectoderm, gave a negative result. I have lately re-examined the last-named species, but again unsuccessfully: particles of carmine suspended in water were taken in large quantity into the entoderm and mesoderm cells; but the ectoderm remained entirely free from them.

From this I can only infer that, however probable a priori the ingestion of food by ectoderm cells in sponges may be, it is at present by no means proved. More favorable objects for these researches are the true Cœlenterates. A digestive ectoderm has, as far as I am aware, been described in only a single one of these. Merejkowsky,<sup>1</sup> in describing a *Bougainvillea* in which the alimentary canal was rudimentary, has put forward the supposition that in this *Medusa* the food was taken in entirely by ectoderm: but he most carefully asserts that he never found solid particles in the ectoderm of the abnormal *Bougainvillea*, which he supposes to be nourished entirely by organic matter in solution in the sea water.

It has long been known that the ectoderm of hydroid polyps protrudes pseudopodia, which frequently anastomose to form a kind of plasmodium; and it occurred to me that these pseudopodia might have the function of picking up food particles. I have, so far, been able to verify this supposition only in the single case of the so-called *nematocalyces* of *Plumularia*. I have worked with *P. setacea*, Ellis, and with a species bearing very large gonophores, and nearly allied to *P. halecioides*.

If powdered carmine be suspended in the water surrounding a *Plumularia*, it will, after some little time, be evident that a

<sup>1</sup> "Sur une anomalie chez les Hydroméduses et sur leur mode de nutrition au moyen de l'ectoderme." 'Arch. Zool. Exp.,' viii, 1879 et 1880.

considerable quantity has entered the substance of the ectoderm of the nematocalyces. I have many times repeated this experiment, and always with the same result. Though one cannot conclude, from this one observation, that the nematocalyces fulfil the function of collecting food, yet the fact was striking enough to invite further investigation. Examination shows that these organs send out from their projecting extremities various kinds of pseudopodia, which attach themselves either to a calyx, or to its contained polyp, or more frequently flatten themselves out round the stem, so as almost to encircle it. The ectoderm cells of these free extremities are not distinguishable one from another, but fuse into a common protoplasmic mass, which sends out some few pseudopodia. The slow, creeping movements of the ends of the nematocalyces probably serve to clean the neighbouring polyps—a function which accounts for the frequent presence of foreign particles in their ectoderm. Striking results are obtained by studying colonies which have been for some little time in a watch glass. Plumularia polyps, like so many of their allies, are very delicate organisms, which only live a short time after gathering. The whole colony does not, however, die, but only the polyp-heads; the cœnosarc and nematocalyces survive, and will, under favorable conditions, produce new polyps. The last-named organs, in these circumstances, serve to eat up the dying hydranths—an operation I have witnessed repeatedly in both the species of Plumularia I have examined. After the polyp has retracted its tentacles, and become a mere rounded mass, the free end of a nematocalyx creeps into the theca, and gradually absorbs, by means of its ectoderm, the whole contents of the cup. So that on the second day after gathering, when most of the polyps have vanished, the ectoderm of the nematocalyces is seen to contain a large number of foreign particles of different kinds.

It is further evident, on examining the loaded nematocalyces, that the ingested material remains in the ectoderm, and is not, as seemed to me possible, passed on to the endoderm. For a few days after this, no marked alteration takes place, so that I can only put it forward as a hypothesis that the ingested

granules are digested in order to provide material for the building up of new polyps. The so-called nematocalyces must therefore be classed among organs whose chief function is prophylactic; they eat up necrotic parts of the colony, and also continually explore the organs in their vicinity, in order to render harmless by devouring them any injurious bodies which may be present. Any offensive or defensive function seems to be purely secondary; at least the larger of my species had no nematocysts in its "nematocalyces."

It seems probable that the peculiar organs described by Weissmann<sup>1</sup> in *Endendrium racemosum*, and the tendrils observed by Fraipont<sup>2</sup> in *Campanularia angulata*, serve the same function as that of the nematocalyces of *Plumularia*.

The Actinias give us another case of the ingestion of solid food by the ectoderm. Three years ago, in my work on the development of *Actinia mesembryanthemum*, I showed that the tentacles of this creature habitually take up a large number of carmine granules. During the past year I have studied the viviparous edible *Actinia* of Pantano (which is identical with the new *Bunodes sabelloides* of Dr. Andres), and I found that the larvæ of this species also contained in their ectoderm a large quantity of foreign matter. The younger the larva, the more abundant were the extraneous granules. One can often find Gastrulæ, whose bodies are asymmetrically swollen, and dirty looking. Examination shows that this dirtiness is not due to particles adhering to the outside, but that it arises from the presence in the ectoderm cells of foreign bodies, sometimes black, irregular or angular, sometimes rounded and fatty or albuminoid in character. The periphery of the ectoderm (which seems from examination of the living larva to have all its cells fused into a common homogeneous ectoplasmic mass) is free from granules, all the ingested matter being in the deeper parts of the protoplasm, either just in front of the nucleus, or behind it. The particles

<sup>1</sup> 'Mittheil. aus d. zool. Stat. zu Neapel,' iii, 1882, p. 1.

<sup>2</sup> 'Recherches sur l'organ. histol. et le develop. de la Campan angul.' 'Arch. Zool. Exp.,' viii, 1879, 1880, p. 442.

are usually embedded in the protoplasm itself; but are sometimes surrounded by a vacuole indicating the occurrence of some digestive process. The ectodermal granules are identical with those existing in the endoderm and in the gastric cavity of the larva, showing that they are not to be considered as products of the metabolism of the organism itself. In fact, we are forced to believe from what has been said that the larvæ of an *Actinia*, when in the body of the parent, are commensal parasites, living on the food taken in by the mother. If the larvæ be removed from the mother, and put into water containing carmine in suspension, the carmine granules are eaten by the ectoderm cells, being seized by means of short pseudopodia extended from the free surface.

After the development of the gastric pouches, the number of foreign particles in the ectoderm is much smaller. Young larvæ, still in the body of the mother, in whose ectoderm no granules can be seen, retain the power of ingesting carmine granules, especially in the ectoderm of the tentacles and disk. It is exceedingly difficult to follow the further history of ingested granules, whether in ectoderm or endoderm; but it is hardly conceivable that they should be ingested with any other object than that of subsequent absorption.

As a further example of ectodermal nourishment, may be cited the ovarian ova of those animals whose generative cells are ectodermal—for example, those of *Tubularia*, and, according to Korotneff, of *Hydra*. In the first-named animal I have seen the young amœboid ovum eat and digest the neighbouring follicular cells. Korotneff<sup>1</sup> asserts, without giving any proof of his statement, that during the winter the young ectoderm cells of *Hydra* devour the older ones.

## II.—INTRACELLULAR INGESTION AND DIGESTION BY WANDERING MESODERM CELLS.

While the taking up of nutriment by ectoderm cells can only be observed in rare and exceptional cases, nothing is easier

<sup>1</sup> 'In his Russian memoir on *Myriothela* and *Hydra*.' Moscow, 1882, p. 43.



than to find amœboid cells of the mesoderm, which both ingest and absorb food particles. It has long been known that sponges are nourished by means of amœboid cells; but the morphological value of such elements was unknown. After I had suggested<sup>1</sup> that they were to be compared to the true mesoderm of other creatures, F. E. Schultze<sup>2</sup> proved, by the discovery of a true ectoderm, and by a careful histological examination of the cells in question, the correctness of my view, which has since been generally accepted. It is also generally believed that these mesoderm cells play an important part in the nourishment of the organism; some observers (Balfour, von Lendenfeld) attributing the function of ingestion to these cells alone, while others allow the endoderm some share in the process. I will not stop to discuss this point, because, in the first place, it is for the moment of no importance to us whether the endoderm ingests or not; and, secondly, in a future paper on the endoderm, I hope to enter fully into the question. I have only mentioned sponges at all in order to remind the reader that ingestion by the mesoderm is an established fact among them—a point which will be of use to us in discussing the higher forms.

Leaving, then, the Porifera, I must refer to the observations on the ingestion of granules of colouring matter by blood-corpuscles among the lower Invertebrates. Haeckel<sup>3</sup> was the first to show that when a *Tethys* is injected with indigo, the granules are taken up by the blood-corpuscles. Later on he proved the occurrence of similar phenomena in the blood of various Invertebrates. Haeckel, however, did not perceive the close analogy between this process and the mesodermic nutrition of sponges; indeed, he subsequently<sup>4</sup> contradicted the statements of Lieberkühn as to the functions of the wandering cells of *Spongilla*. His observations on the blood-corpuscles became

<sup>1</sup> "Zur Entwicklungsgesch. d. Kalkschwämme," 'Zeitschr. f. wiss. Zool.,' xxiv, p. 10.

<sup>2</sup> "Ueb. d. Bau u. d. Entwickl. von *Sycandra Raphanus*," 'Zeitschr. f. wiss. Zool.,' xxv, suppl., p. 258.

<sup>3</sup> 'Radiolarien,' 1862, p. 104.

<sup>4</sup> 'Die Kalkschwämme,' 1872, Bd. i, p. 372.

the starting-point of a large series of important researches in vertebrate histology and pathology ; but by the pure zoologist they have remained unnoticed.

The ingestive and nutrient functions of wandering mesoderm cells are very various.<sup>1</sup> Their importance in the resorption of parts which have become useless or harmful has already been alluded to. I have been able to observe this property most easily in Echinoderm larvæ, especially in the *Auricularia* of *Synapta*, and in the so-called *Bipinnaria asterigera*. In both these forms large numbers of amœboid cells appear between entoderm and ectoderm, giving rise to all the skeletal structures, and the cutis of the adult, and to the oral musculature of the larva. Their function, however, is not purely morphogenetic. At the period of metamorphosis, which is, as is well known, extremely complicated, and associated with the loss of many larval organs, these mesoderm cells ingest the cellular débris of the disappearing organs, and finally absorb them. In *Auricularia* numbers of these amœboid cells collect beneath the ciliated rings just before metamorphosis, because in this region the phenomena of resorption are most pronounced. The ciliated cells then break down into albuminoid globules, which are devoured by the amœboid elements below. *Auricularia* is so transparent, and so easily obtainable, that it is not difficult in this form to watch the process of ingestion and absorption of débris in a single cell. The albuminoid granules may remain for some time in contact with the amœboid cells, lying on their pseudopodia, and then be suddenly swallowed ; or the swallowing process may be so gradual as to allow of the various stages being seen and drawn. The absorption, like the ingestion, of these granules seems to vary greatly in rapidity ; in some cases it commences at once and is soon completed, while in others one can watch an ingested granule for hours without noticing the slightest change.

Resorption phenomena, similar to those just described, can

<sup>1</sup> I may here remark that by "wandering mesoderm cells" I mean the so-called amœboid connective-tissue cells, as well as lymph- and blood-corpuscles.

be seen at two stages in the life-history of *Auricularia*. They first occur at the assumption of the so-called pupa stage, when a large part of the longitudinal ring of cilia is lost—that is, is disintegrated and devoured by the mesoderm. At this time every amœboid cell of the middle layer is generally loaded with enormous numbers of débris granules, which are slowly absorbed during the pupa stage, so that the cells which contained them become filled with clear vacuoles. On the metamorphosis of the pupa into a young *Synapta*, the cells begin again their devouring work, collecting as before beneath the ciliated rings, and eating up the products of disintegration. The appearances seen during the process at this time are exactly those seen at the time of its first occurrence.

Similar phenomena can be observed in *Asterid* larvæ, where large tracts of larval tissue atrophy during the metamorphosis. In this case, also, the disintegrating cells break up into albuminoid granules of various sizes, which are gradually eaten and absorbed by mesodermal elements.

I have found these appearances so constantly to accompany metamorphosis, that I cannot but regard them as normal and necessary events in the life of an Echinoderm larva; and I am therefore forced to the conclusion that the wandering mesoderm cells of which I have spoken play the same part in the resorption of larval organs as that played by osteoclasts in the resorption of Vertebrate bone. I have never seen, however, in Echinoderm larvæ, any formation of multinuclear masses similar to those seen during the resorption of bone.

It is hardly possible to believe that this resorbent function of the mesoderm should be confined to Echinoderms. I rather incline to the belief that it occurs in all animals whatever which undergo any great degree of metamorphosis. I have reason to believe that wandering cells play an essential part in the complicated larval changes of Ascidians. I have not, indeed, been able to prove this in the case of *Ascidia intestinalis*; but only, I believe, because of the small size of the cells in question; but I have frequently seen wandering cells loaded with débris. If this should prove to be the case, we should have

a simple explanation of such appearances as the transformation of the degenerating nervous system into a heap of blood-corpuscles, which is at present believed to be due to a direct morphogenetic change in the ganglion cells.

It may here be pointed out that Ganin, in 1876, attributed to amœboid mesoderm cells an important part in the so called histolysis of muscle-fibres in flies.<sup>1</sup> He says: "During the early stages of development I have often seen free amœboid mesoderm cells in the cavity of the foot attach themselves to the surface of a muscular mass, into which they often bored deeply, appearing to nourish themselves from the substance of the larval muscle." Viallanes, the latest student of histolysis,<sup>2</sup> has not sufficiently considered Ganin's observations. Though the very few drawings which he gives point strongly to the conclusion that there is a considerable ingestion of food by mesoderm cells during metamorphosis, he expresses himself in his text in favour of a totally different view. Thus, he describes as "degenerated blood-cells, like pus-corpuscles," elements which have probably only loaded themselves with albumin granules; while his "cellules musculaires," arising out of muscular débris, are also, in all probability, nothing but overloaded mesoderm cells.

Though this ingestive power of the mesoderm is specially pronounced during metamorphosis, it occurs on other occasions. In 1880 Schneider<sup>3</sup> showed that resorption of the generative products by amœboid cells resembling blood-corpuscles occurred in Hirudinea; an observation which I have repeated for *Aurelia aurita*. Many of the ovarian ova of this medusa become surrounded by amœboid cells, and completely devoured. These cases can be compared with the following, which have been observed by me. If a *Pilidium* be left for some time in a watch-glass, the rudimentary nemertine

<sup>1</sup> 'Beiträge zur Kenntniss der postembryonalen Entwicklung der Insecten.' Warschau, 1876 (Russian).

<sup>2</sup> "Recherches sur l'histologie des Insectes." 'Ann. Sci. Nat.,' v, xiv, 1882, pp. 135—158.

<sup>3</sup> "Ueber die Auflösung der Eier und Spermatozoen in den Geschlechtsorganen." 'Zool. Anz.,' 1880, p. 19.



atrophies, being devoured by amœboid mesoderm cells around it; so that there remains in the watch-glass an apparently normal *Pilidium*, only with its rudimentary nemertine replaced by a number of amœboid cells, full of food-granules.

In all these cases the material eaten by mesoderm cells has been formed from the body of the animal itself, and has only become useless at the moment of its being devoured. These cells can easily be proved capable of ingesting, and usually of digesting, material altogether foreign to the organism in which they live. If a large number of transparent creatures (larval or adult), which possess a mesoderm, be taken fresh from the sea, it is easy to find, among a number of empty mesoderm cells, some containing foreign particles which may be of very different kinds. In the mesoderm cells of Echinoderm larvæ I have often found empty thread-cells. Similar structures can often be found in Ctenophora and *Pilidium*. I believe that bodies such as these, when found in the mesoderm cells, have pierced through the body wall, and then been swallowed. Just beneath the epidermis there is generally present a whole multitude of amœboid cells, ready to take up anything which may pierce the body wall. In my early investigations on the intra-cellular digestion of Ctenophores<sup>1</sup> I saw that carmine granules suspended in water passed, not only into the endoderm cells, but also into those of the mesoderm; though I could not then determine the exact mode of their entry.

These phenomena—on the one hand the process of resorption, on the other the frequent enclosure by mesoderm cells of foreign bodies—show how extremely well developed is the power of ingestion and absorption in these cells. I have, therefore, made several experiments with the object of defining more clearly the extent of this property. I chose for this purpose *Bipinnaria asterigera* and *Phyllirhœe bucephalum*, because these animals are not only transparent, but large enough to admit of the performance upon them of simple

<sup>1</sup> "Ueber die intracellulare Verdauung bei Coelenteraten." 'Zool. Anz.,' 1880, p. 262.

operations, which they are also hardy enough to survive for some time.

If water holding carmine or indigo in suspension be injected beneath the epidermis of the animal under observation, the particles of colouring matter are after a very short time taken up by the amœboid cells. In *Phyllirhœ*, there are two kinds of amœboid cells, of which only the smaller ingest colouring matters in this way. The larger cells which often assume very curious forms, are distinguishable by their vacuolated protoplasm, and by containing no foreign bodies; in a *Phyllirhœ* which had been injected with powdered carmine, these large cells contained rosy patches, caused by dissolved carmine. In spite of repeated trials, I could not ascertain the way in which the carmine entered them. The smaller granules of solid carmine were all eaten by the small cells, in a manner precisely similar to that already described; the larger masses were, on the other hand, surrounded by a sort of plasmodium of small cells, which came one by one to each lump, and flattened themselves upon it, fusing with neighbouring cells as these arrived. In this way arose plasmodia of very different sizes, some even visible to the naked eye, which may be compared to the giant cells so often described in Vertebrates. This observation, which I have often repeated, confirms the opinion of Weiss,<sup>1</sup> Koch,<sup>2</sup> and others, that giant cells are often found in the neighbourhood of foreign bodies. In all cases in which I have found giant cells in Invertebrates, they have arisen round foreign bodies, and always by the fusion of separate cells. My results are therefore opposed to the view, held by some pathologists, that giant cells are formed by absorption of pus cells, or by a process of incomplete fission, arrested after division of the nucleus. Other observers, who have studied Invertebrate blood-corpuscles outside the body, have described their great tendency to form plasmodia, without the presence of foreign particles.

<sup>1</sup> "Ueber die Bildung und die Bedeutung der Riesenzellen, &c.," 'Virchow's Archiv,' Bd. lviii, p. 13.

<sup>2</sup> 'Berliner klinische Wochenschrift,' 1882, No. 15.

Haeckel<sup>1</sup>, for example, has observed this in Echinoderms, and Geddes<sup>2</sup> in the lymph cells of *Lumbricus*, in Mollusks, and in *Pagurus* and other Decapods. These observations are entirely confirmatory of the views of those observers who regard giant cells as true plasmodia, due to a fusion of several distinct cells.

Another example of the formation of mesodermal plasmodia (as I shall henceforward call giant cells) was seen in an *Asterigera*, which had had a drop of human blood injected beneath the skin. Most of the corpuscles were ingested, each by one or two separate cells; but the larger clots were each surrounded by several cells, which fused to form plasmodia. These latter, though loaded with masses of corpuscles, were yet able to move by means of large pseudopodia. The nuclei were so obscured that it was impossible to observe them in the living state; I therefore treated the plasmodia with alcohol and borax carmine, finally clearing with oil of bergamot. By this treatment the nuclei came out distinctly; they were all situated in the peripheral part of the plasmodium, the centre of which was filled with great balls of fused blood-corpuscles.

We should conclude, from the above-described observations, that when mesoderm cells are confronted with a large mass of food material, which they cannot devour singly, they fuse into a plasmodium, which eats up the whole available food. This is not, however, invariably the case. When the mucous tissue of *Phyllirhœ* was filled with large bodies, such as the boiled eggs of *Sphærechinus granularis*, or boiled cells from the cotyledons of peas, I could see no formation of plasmodia. Shortly after the introduction of these bodies into the tissue, small amœboid cells collected round them in great numbers, and remained for several days—till the death of the animal—closely surrounding them, but without the slightest sign of fusion one with another. The cells surrounding the tissue of the pea remained inactive during the whole time; for, being unable to perforate the thick cellulose wall, they could not commence in-

<sup>1</sup> 'Radiolarien,' p. 103, Anns. 2.

<sup>2</sup> "On the Coalescence of Amœboid Cells into Plasmodia," 'Proc. Roy. Soc.,' 1880, p. 252, pl. v.

gestion. Those around the echinus egg, on the other hand, became filled with small particles of yolk, while yet retaining their complete independence one of another. I have seen the same thing occur on introducing large bodies, such as glass spicules, rose thorns, echinus spines, &c., beneath the skin of *Bipinnaria*, *Tethys*, and *Terebella*, and into the deeper layers of the mantle of *Ascidia intestinalis*. Soon after the foreign substance appeared within the body, the amœboid cells (connective-tissue corpuscles in *Bipinnaria* and *Tethys*, lymph corpuscles in *Terebella*, test-cells in *Ascidia*) began to collect around it, finally surrounding it completely, and forming a mass so large as to be easily visible to the naked eye, while remaining perfectly distinct from one another, and so not forming a true plasmodium. In *Tethys* I once saw a partial fusion of cells into a complex, the component elements of which were still, however, easily recognisable. It was perfectly easy, by means of sections to make sure of the absence in *Tethys* of true plasmodia in these "inflamed" regions (I made use of the ear-shaped tentacles in my observations).

It follows from this, that while mesoderm plasmodia, when they arise in the animal body, are formed round foreign substances, yet that this formation is not a necessary consequence of the presence of such substances, it being perfectly possible for the reaction of the organism against intruded matter to take place without any such formation. It has also been shown that one function of amœboid mesoderm cells is to eat up those parts of the organism which have become useless, and also any foreign bodies which may have pierced through the ectoderm; or, if it be not possible to eat up such bodies, to surround and isolate them. It is obvious that the process of removal of small masses of detritus, or minute grains of carmine, on the one hand, is fundamentally identical with that of surrounding larger foreign bodies, on the other. Glass rods, atoms of dust or carmine, are surrounded or devoured by aggregates of cells in exactly the same way. It is also undeniable that the results of introducing a glass spicule, or other irritant, into the body of an Invertebrate, bear no small resemblance to



the phenomena of inflammatory exudation in Vertebrates. In both cases a number of mesoderm cells collect round the irritating body, and act upon it as best they may. The difference between the two cases is only one of degree. In *Bipinnaria*, which has as yet no trace of a vascular system, we see a gradual accumulation of the numerous amœboid cells, which are scattered throughout the mesoderm; while in molluscs the lacunar blood-vessels play a purely passive part in allowing the corpuscles to flow through them. In *Terebella*, with its closed blood system and red plasma, inflammation<sup>1</sup> affects only the so-called lymph corpuscles of the body cavity; if the blood-vessels be not injured by the intrusive body, no transudation occurs, as is evident from the absence of coloration. Therefore, from the point of view of comparative pathology, Cohnheim's dictum, "without vessels no inflammation,"<sup>2</sup> which has been accepted by many pathologists, does not hold. Inflammation is a phenomenon phylogenetically much older than blood-vessels, while exudation is a comparatively late development. From this point of view, it is evident that the white corpuscles of Vertebrates must be regarded as of more importance than has been thought to be the case, thus justifying the views of Thoma.<sup>3</sup>

Our observations on resorption during metamorphosis among Echinoderms (which are in complete harmony with the results of histological and pathological investigation on Vertebrates<sup>4</sup>) have taught us that mesoderm cells are able to take up and to digest albuminoid granules. This conclusion is strengthened by the following observations.

If we follow the fate of (human) blood-corpuscles, after their

<sup>1</sup> I have so far had no opportunity of examining the phenomena of inflammation in those annelids which possess well developed blood-corpuscles; I hope shortly to fill up this gap in my observations.

<sup>2</sup> "Ueber Entzündung und Eiterung," 'Virchow's Archiv,' 1867, Bd. 40; compare also his 'Neue Untersuchungen über die Entzündung,' Berlin, 1873, p. 11, 42, 62, 67, 71.

<sup>3</sup> "Ueber Entzündliche Störungen des Capillärkrieslaufs bei Warmblütern," 'Virchow's Archiv,' Bd. 74, p. 386.

<sup>4</sup> Summarised by Ziegler in his 'Lehrbuch der pathologischen Anatomie,' 2 Aufl., Bd. i, 1882, pp. 167—175.

ingestion by the mesoderm cells of *Bipinnaria*, we see that they are completely absorbed. Within the cell they swell up and become clearer; the hæmoglobin is then dissolved out, and finally the whole corpuscle disappears. (I need hardly say that corpuscles which have not been eaten do not undergo this series of changes.) The corpuscles of *Discoglossus*, injected into *Phyllirhœ*, behave somewhat differently. The cell-body, and the nucleus, of such a corpuscle, becomes somewhat irregular in shape after ingestion; a crumpling process goes on, which results in the breaking up of cell-body and nucleus into several fragments. At this stage the central part of the mesoderm cell becomes slightly coloured. The whole process resembles the resorption within the so-called blood-corpuscle-containing cells of Vertebrates, which must really be regarded as a process of feeding on the part of a mesoderm cell.<sup>1</sup>

Milk injected beneath the skin of *Bipinnaria* and *Phyllirhœ* has the same fate. The milk spherules are eaten by wandering cells, lose their shining appearance, and break up into small granules, which are distributed throughout the cell substance. I have not yet observed any noticeable alteration in ingested starch grains.

In order to ascertain whether the mesoderm cells exercised any choice in the particles they absorbed, I injected mixtures of different kinds. For example, I filled the mucous tissue of a *Phyllirhœ* with a mixture of milk and indigo, carmine and starch grains—nutritious and useless bodies together—and I found that all these bodies were equally absorbed, some cells eating all four kinds of food at the same time. From this it would be supposed that the mesoderm cells ate everything provided for them, without power of distinction. The following experiment, however, seems to contradict such a view. On injecting into a *Phyllirhœ* living ovarian ova from a *Sphærechinus granularis*, it was found that neither young ovarian cells, nor ripe ova which had extruded polar bodies, were eaten by the mesoderm cells; on the contrary,

<sup>1</sup> G. Langerhaus "On the Resorption of Extravasations and the Formation of Pigment within them," 'Virchow's Archiv,' 1870, Bd. 49, pp. 81, et seq.

ova in all stages seemed to live much longer when taken from the ovary and placed within the tissues of *Phyllirhœ*, than when simply placed in sea water. My observations lasted on one individual six days; that is, until its death. I was also able to fertilise these eggs within the *Phyllirhœ*, normal segmentation and a normal blastosphere being produced. If, however, boiled eggs of *Sphœrechinus* were introduced, amœboid cells immediately fastened upon them, and began to devour their yolk. This might be taken as proof that the amœboid cells eat only dead matter, were it not that in the cases already described, of the ingestion of red blood-corpuscles, some, at least, must have been alive when they were surrounded or devoured. As a further experiment, I introduced a drop of living semen of *Sphœrechinus granularis* beneath the skin of *Phyllirhœ*. The spermatozoa slackened their movements, and were soon surrounded and eaten by the mesoderm cells. A few remained for two days undevoured, and retained their power of fertilisation.

So that the amœboid cells do not take up everything that is offered them, and we probably should not deny their possession of some means of distinguishing between desirable and undesirable substances. But why they made no attempt to attack the living ova in the case described, seems at present inexplicable.

It has been sufficiently proved, that the cells of the mesoderm have important prophylactic functions; a result which invites further investigation. Observations on necrotic organs of several Invertebrates, especially of *Bipinnaria asterigera*, have shown it to be one function of the mesoderm cells to devour the dying elements of such organs. The long arms of *Bipinnaria* end in orange coloured points, covered by an ectodermal pigmented epithelium, and containing more or fewer mesoderm cells. These latter generally contain rounded pigment granules, derived in all probability from the ectoderm. When the animal has been for some time in a watch-glass, the ends of the arms become worn out and broken, so that large pieces frequently fall off. In the ectoderm of such unhealthy arms are numbers of rounded granules, which

are eaten by mesoderm cells, just as are the similar granules, found during metamorphosis; so that the amœboid cells of the arms are often crammed with débris. In addition to the clear, feebly refracting degradation products, one often finds in the ectoderm of *Bipinnaria* masses of small, round, strongly refracting bodies which are devoured by the mesoderm cells. I have not been able to determine exactly the nature of these bodies, but it seems possible that they may be spores of bacteria, since they closely resemble undoubted spores, while it can be shown that the mesoderm cells will readily ingest such bodies. If fluids containing bacteria be injected beneath the skin of *Bipinnaria* or *Phyllirhœ*, or if they develop spontaneously in the wounds of such animals, they will soon be found within the substance of many amœboid mesoderm cells. Both still and motile forms are thus ingested, and they may be found either embedded in the protoplasm of the absorbent cell, or surrounded by a vacuole. Individual bacteria are often seen which retain their power of movement even after ingestion, while in other cases the motility is lost at once, and the whole bacterium becomes so delicate as to be scarcely visible. We may then consider that bacteria are habitually ingested by mesoderm cells, when they make their appearance in the organism;—a fact which obviously increases the prophylactic importance of these cells. These phenomena can perhaps be most easily seen in *Botryllus*, colonies of which, when freshly gathered, contain almost invariably large quantities of bacteria within the test; I found especially a *Spirochœta*, closely resembling the *S. Obermayeri* of relapsing fever, and a small *Bacillus*, like the *Lepra bacillus*, which had a spore at each end. Both these forms were pursued by the wandering cells of the *Botryllus*, and were found ingested and absorbed by them in various stages of development. The victory was not, however, all on one side; here and there were found mesoderm cells to all appearance dead, with long bacterial filaments projecting from them.

The same thing has been observed in Vertebrates, where



Koch has found both *Bacillus anthracis* and the bacillus of septicæmia in the mouse enclosed by white blood-corpuscles,<sup>1</sup> while tubercle bacilli have been seen by him in the interior of giant cells.<sup>2</sup> So that throughout the whole animal kingdom the wandering cells of the mesoderm make use of their ingestive power for the destruction of bacteria and similar organisms, which need for their development a suitable (necrotic) nidus. In Metazoa with an undeveloped mesoderm, this function is performed by the ectoderm (*Plumularia*) or by the endoderm.

Most embryologists agree that the Metazoa are to be derived from ancestors closely resembling the colonial Monads. Now, the individuals composing these colonies are all exactly similar one to another, so that we can find among them no trace of that division of labour which is the first step in the differentiation of germinal layers. There is even less differentiation among the colonial Protozoa than among their chlorophyll containing allies, the Volvocineæ. The attempt to form some exact idea of the origin of the lowest Metazoa, as the basis of a comparative biology, is, therefore, extremely difficult. Observers are agreed in considering the blastula stage to represent an ancestral colony of Monads; but they differ fundamentally in the meaning attached to the formation of the germinal layers. Some, as, for example, Balfour, unhappily so early lost to science, suppose that the blastula cells (or rather, the individuals composing the colony which this stage represents) were early differentiated into two kinds; so that the transitional form between Protozoa and Metazoa consisted of a hemisphere of nutrient amœboid cells, joined to a hemisphere of ciliated locomotive cells.<sup>3</sup> I have supposed that some of the blastula

<sup>1</sup> 'Unters. ueb. d. Aetiologie d. Wundinfektionskrankheiten,' 1878, pp. 44, 72. Koch's opinion that bacteria force themselves into the white corpuscles in order to multiply there does not seem proved, and does not agree with my results.

<sup>2</sup> 'Berliner klinische Wochenschrift,' 1882, No. 15. Zopf's interpretation of Koch's observations does not seem probable ('Die Spaltpilze,' Berlin, 1883, p. 67).

<sup>3</sup> " . . . The larva of Sponges is to be considered as a colony of Protozoa,'

cells, which, like all the others of the colony, had the power of obtaining and ingesting food, travelled from various parts of the periphery of the blastosphere to the interior, losing their columnar character, and becoming amœboid. These amœboid cells I regard as the rudiment of a parenchymatous endomesoderm, which afterwards differentiated into true endoderm and other structures.<sup>1</sup>

The discovery of ectoderm cells, which retain the power of ingesting food, in various groups of Cœlenterates, seems to me totally incompatible with an early differentiation of the blastula cells, such as is assumed by Balfour, and rather goes to show that at the stage in question the whole ectoderm habitually performed those functions which are now limited to the endoderm. It is very difficult to suppose that so fundamental a property as the nutrient power of a Monad cell should have disappeared in so short a time as that occupied by the differentiation of the two halves of the ancestral *Sycandra*.

Further, I am aware of no reason, embryological or otherwise, which prevents our assuming that the germinal layers were differentiated at a time when all the cells of the organism retained the power of taking up food. Later on, this power was restricted to the parenchyma cells, as in Sponges, where the food is taken in by a parenchymatous mesoderm, which cannot be clearly distinguished from the endoderm, as even in the adult cells are continually passing from one layer to the other. Only later in phylogeny was a sharp distinction established between the two divisions of the primitive parenchyma, or, as it may be called, phagocytoblast. But in the

one half of the individuals of which have become differentiated into nutritive forms, the other half into locomotor and respiratory forms. . . . That the passage from the Protozoa to the Metazoa may have been effected by such a differentiation is not improbable on a priori grounds." 'Comparative Embriology,' i, p. 122. [This is quoted from the English edition. In the German translation, quoted by Metschnikoff, Balfour is made to assume the passage of all Metazoa through a form like that of the *Sycandra* larva, an opinion which he certainly never expressed.—TRANSLATOR.]

<sup>1</sup> "Spongeologische Studien," 'Zeitsch. f. w. Zoologie,' 1879, Bd. xxxii, p. 375, et seq.

lower Cœlenterates we can even yet hardly speak of a mesoblast. Though many Acraspeda have amœboid cells in their gelatinous tissue, others have no trace of such elements; and their presence in Craspedota is altogether exceptional. It is worthy of note that in Medusæ these cells, when present, appear only late in life. If we consider the supporting cells of the tentacles in Medusæ and hydroids as mesoderm, we are still unable to draw a line between them and the endoderm.

In higher forms, the distinction between the two layers is much clearer; the endoderm has assumed the function of utilising the food brought to it from without, and an intracellular absorption is gradually replaced by a process of enzymogenesis (a change which will be more fully discussed in the third section of this work). The mesoderm does not, however, lose its primitive powers, but employs them against useless and harmful bodies, so that it retains the intracellular digestion, as well as many other of the characters of the Protozoa—not only the power of throwing out Pseudopodia, but also that of forming Plasmodia. This last property has persisted less in the ectoderm than in the other layers, being only seen in the epidermis of Sponges, Hydroids, and perhaps a few other Cœlenterates; it is well preserved in the endoderm of animals with an intracellular digestion, such as Cœlenterates and Turbellarians. Mesodermal Plasmodia are, however, found even in the higher animals, not excepting Man himself. The cells of the mesoderm have best preserved their primitive independence one of another, so that one can truly say that the protopsychic condition here persists.

Many embryologists, in their endeavours to elucidate the history of the middle layer, have attempted to determine its special function. Some (Hatschek) have believed it to be specially connected with reproduction, while others (Rabl) consider that it arose in connection with a locomotive apparatus. Considering the facts described in this paper, and, above all, if we remember that in many animals with a mesoderm both generative organs and musculature are derived, not from this

layer, but from the ectoderm or endoderm (generally, perhaps, from the ectoderm), it is evident that the primitive function of the mesoderm must be nutritive, and its relation to the various tissues and organs purely secondary. Apart from any other argument, I would point to such a creature as *Halisarca*, where there is no musculature at all, and where the greater part of the mesoderm is entirely given up to the performance of nutritive functions.

When I speak of the phagocytoblast, as a whole, I do so because development shows us how intimately the mesoderm, or, at least, the greater part of it, is connected with the endoderm. Apart from the facts in the development of Sponges, which I have elsewhere described, I will mention a few points in the formation of the amœboid mesoderm cells in Echinoderms, *Pilidium*, &c., which support this view. But I must state, at the outset, that I do not exclude the ectoderm from all share in the formation of the middle layer. I would rather suppose that, in earlier times, when the ectoderm had not so completely lost its ingestive power, and when the phagocytoblast was still partly derived from it, amœboid cells were frequently budded off from the ectoderm to join the other devouring cells (phagocytes) in the body. In this way may be explained the ectodermal origin of a part of the mesoblast in the larva of *Halisarca*.

We must also bear in mind that other elements, besides phagocytes, acquired a secondary connection with the mesoderm, such as reproductive, muscular, and other cells. So that what we call mesoderm is really a heterogeneous mixture of elements acquired from various sources at various times; and the origin of the whole of these, as a single germinal layer, must be regarded as a comparatively late event. In this view of the mesoderm I agree with Balfour<sup>1</sup> and the brothers Hertwig.<sup>2</sup> In speaking, however, of the mesoderm as a nutritive cell-complex, I have done so because I regard this as its primitive and most important function. A detailed phylogenetic history of the mesoblast throughout the Metazoa seems at present im-

<sup>1</sup> 'Comparative Embryology,' vol. ii, p. 286.

<sup>2</sup> "Die Actinien," 'Jenaische Zeitschrift,' 1879, vol. xiv.



possible; but whether this be so or not, I am convinced that such a history can never be obtained by purely morphological and histological methods, such as those employed by the Hertwigs in their "*Cœlomtheorie*."

Since we have seen that the power of intracellular ingestion and absorption is used as a protection against harmful bodies arising within an organism on reaching it from without,<sup>1</sup> it follows that septic organisms (*Bacteria*, *Chytridæ*, *Entomophthora*, and other parasites) are a very old source of trouble in the world; and perhaps many organs and events, whose significance has hitherto been overlooked, may find an explanation in this way. I would especially mention the nematocalyces of *Plumularia*, and the peculiar test of *Ascidians*. It is evident that instances of prophylactic organs will soon be multiplied, so as to far exceed in number the few I have given. Here is yet another case. On the inner surface of the contractile walls of the excretory organ of *Carinaria* there are certain "granular cells," in which a formation of renal concretions is believed to occur, as in the *Gastropod* kidney. I have, however, been able to prove that these concretions are not formed in the cells, but are really foreign particles taken up by the cells, which are amœboid, as may be proved by suspending carmine or indigo in the water in which the *Carinaria* lives. Since the excretory organ pumps water into the pericardial cavity, these cells are posted on its walls to prevent the entrance, with the water, of bodies harmful to the organism.

The great advances made in Pathological Science during the past few years cannot fail to benefit pure Zoology, which will, in its turn, help to solve the problems of Medicine by establishing a Comparative Pathology, based on the doctrine of Evolution.

<sup>1</sup> In this way may be explained the recent observations of Buchner on the action of inflammation on *Bacteria* ('*Die ätiologische Therapie und Prophylaxis der Lungentuberculose*,' Müschen, 1283, pp. 11 and 12).

## The Ancestral History of the Inflammatory Process.

By

**Dr. Elias Metschnikoff.<sup>1</sup>**

UNDER the name of "phagocytes" I have recently described certain cells which have the power of ingesting and sometimes of absorbing food particles. This character of the mesoderm is most marked where we find a number of amœboid cells devouring dead or degraded elements of the body of the animal in which they live. Pathology already furnishes numerous examples of the exercise of this function by white blood-corpuscles; only it has not been recognised that the ingestion of foreign matter by a blood-corpuscle is a true act of feeding, and that the absorption of such a body (a red corpuscle, for example) is comparable to digestion. The results of investigations of Invertebrates, and the fact that in Sponges a great part of the whole function of nutrition is performed by amœboid cells of the mesoderm, while in *Bipinnaria*, *Phyllirhœe*, &c., such cells function indirectly as absorbent organs, made it probable that a similar intracellular absorption would be found in the Vertebrate mesoderm.

The tail of Batrachians is an object well adapted for proving the truth of this supposition. During the early stages of its absorption it contains a large number of amœboid cells, within which are seen remnants of nerve-fibres and muscle cells. These cells may be seen in any quantity by simply teasing up in serum or aqueous humour a piece of any tail in which atrophy has commenced. Left undisturbed for some little time, each cell throws out a number of fine, radiating pseudo-

<sup>1</sup> "On the Mesodermic Phagocytes of Certain Vertebrates," 'Biologisches Centralblatt,' iii, 18, 15th November, 1883.

podia, till it acquires a superficial resemblance to an Actinophrys. I have only seen these phagocytes in the living uninjured tail, in the case of *Bombinator* larvæ, where, at the beginning of the metamorphosis, they collect round the muscles of the tail, the fibres of which are gradually surrounded and devoured. The fragments of muscle retain their structure for some time after ingestion; gradually, however, they lose their striation and break up into rounded strongly refracting globules.

In the body-cavity of Batrachians I found during metamorphosis a large number of similar amœboid cells, which, however, did not contain muscle fragments, but only rounded granules. I think it justifiable to conclude from this that the phagocytes, after feeding on the tissue of the tail, pass into the body-cavity, whence they enter the lymphatics, and finally reach the blood.

The atrophy of the gills is not so easy to follow; but during its progress it is easy to ascertain the presence of large fully laden phagocytes.

So that phagocytes seem to play a part in the metamorphosis of Batrachians as important as that which they have been shown to take in the larval changes of *Bipinnaria* and *Auricularia*. There is also pathological evidence to prove their agency in the so-called active degeneration of muscles and nerves.

In order to ascertain whether in Vertebrates as well as in Invertebrates the phagocytes had the power of ingesting parasitic bacteria, I injected putrescent blood beneath the skin of a frog, so as to induce septicæmia. After a time the white blood-corpuscles were found to contain both still and motile bacteria, each surrounded by a vacuole. The bacteria were especially abundant in the phagocytes of the spleen, which confirms the statement of pathologists that the white corpuscles, when they have ingested an insoluble body, are carried into the spleen. This fact seems to indicate that the spleen is a prophylactic organ, whose function it is to provide for the removal of septic bodies from the organism; that in fact it is in function analogous to the nematocalyces of *Plumularia*.

Bacelli's observation, that fresh spleen pulp can dissolve coagulated milk,<sup>1</sup> supports this view. The well-known fact that many animals can exist for some time after excision of the spleen harmonises completely with the view that this organ plays no important physiological part in healthy life, being merely a weapon against septic bodies (such as bacteria germs especially). It would be interesting to test the relative power of resistance to disease germs in animals with the spleen excised, and uninjured individuals of the same species; though we must remember that other organs, for example, lymphatic glands and marrow, may to a less extent have the same prophylactic power.

Since my researches into the phenomena of inflammation among Invertebrates led me to believe that the whole process was primitively nothing more than a collection of phagocytes assembled to devour the exciting object, I was anxious to see how far the Vertebrates justified this view. I found the most suitable object for investigation was the caudal fin of Triton and other amphibians. By touching a point of the tail with a small piece of nitrate of silver, and then washing with salt solution, it is easy to watch in detail the inflammatory changes.

The well known alterations in the capillaries are much less marked in Triton than in the frog; probably because, the vessels being so much thinner, the large corpuscles can pass through them with greater ease. On the other hand, the Triton larva is a most favorable object for the study of the changes in the connective-tissue cells which collect round the inflamed spot, and eat up the exciting particles. I have seen the branched connective-tissue cells, for example, eat up blood-corpuscles, carmine granules, and particles of pigment. In cases where the cells take up only small amounts of foreign matter, they retain their stellate appearance, the only visible changes being in some of the finer pseudopodia; when, on the other hand, there are large masses to be devoured, the fine processes

<sup>1</sup> "Studien ueber die Funktionen und die Pathologie der Milz," 'Virchow's Archiv,' Bd. 51, 1870, p. 141.



are withdrawn, and the whole cell, losing its normal shape, becomes very actively amœboid. As a result of my observations I am led to believe that no sharp line of demarcation exists between the so-called fixed or stellate and the wandering cells. After ingesting as much as possible of the foreign substance, the connective tissue cells withdraw their pseudopodia and appear as rounded masses.

These observations show that the cells of the connective tissue in the larval Triton's tail must distinctly be regarded as phagocytes, and that they act as such during inflammation. I have several times observed a multiplication of these elements in an inflamed Triton larva: but the process, which can easily be followed in the living subject, with all its associated nuclear changes, happens so seldom that it can hardly play an important part in normal inflammation.

In the frog tadpole the extravasation of white blood-corpuscles is much more important than in Triton. My observations confirm those of several investigators, who assume, as Von Recklinghausen has recently done, an active wandering on the part of the corpuscles themselves, effected by the protrusion of numerous pseudopodia, similar to those extruded by the resting corpuscles of many Invertebrates.

In the frog larva, also, the phagocytes collect round the point of stimulation, and eat up as much of the exciting object as they can (I used in stimulating tadpoles a fine glass tube, filled with cinnabar or carmine). In this state they often remain for days or even weeks. When a fully gorged phagocyte dies, it is immediately devoured by another, so that one can often see a single large cell, containing one or two dead phagocytes, whose nuclei have already disappeared: there is, however, no formation of true multinuclear plasmodia, which indeed I have very seldom seen in any amphibian larva.

I must now point out two results of my observations. First of all, that the most copious diapædesis in inflamed tadpoles was not in the immediate vicinity of the glass tube, but at some distance from it; and secondly, that I never saw any tendency towards a definite aggregation of transudation pro-

ducts, but always an accumulation of phagocytes: so that the so-called serous exudation must be regarded as a secondary result of inflammation, the original result being simply an accumulation of phagocytes.

My whole series of observations, on Vertebrates and Invertebrates together, is hardly compatible with the current theory, which regards inflammation as primarily due to a morbid condition of the walls of the blood-vessels. I rather believe that the essence of the whole process is a struggle between the phagocytes and the septic material, whether the latter be a dead or dying cell, or a fungus, or other foreign body. In Invertebrates, where phagocytes are plentiful, the reaction occurs without the participation of the vascular system—which only comes into play among Vertebrates, where the extra-vascular phagocytes are insufficient. The attraction of the white blood-corpuscles is effected, I believe, by the connective-tissue cells and by the cells of the vascular endothelium, which are known to possess a certain amount of mobility. The first effect of irritation is on the connective-tissue cells, which, as has been shown, are not merely passive during inflammation; and the changes resulting in these may be easily conceived capable of influencing the living cells of the capillary walls, and of inducing such a condition as to favour not only the active transit of the white, but the passive diapedesis of the red corpuscles. We may, therefore, assume the existence of a living chain between the point of irritation and the blood-vessels, which renders possible the intervention of the hæmophagocytes even when, as in keratitis, the inflamed spot is far from a blood-vessel. The links of this chain are (1) connective-tissue phagocytes; (2) endothelial cells of the blood-vessels; and (3) white blood-corpuscles.

If the view here advanced were false, and if the theory, which regards the immediate cause of inflammation as a lesion of the capillary walls, were true, then we should expect to find that in cases where the irritant body occurs in the blood itself, the exudation would not be discontinued, but that the white corpuscles would wander away from the irritant. Such a case is afforded by those diseases which are due to

bacteria. In these, as, for example, in the above-mentioned septicæmia of the frog, where the vessels were directly irritated by numerous bacilli, we find no visible extravasation. The white corpuscles, without leaving the vessel, attach themselves to the bacteria in the blood, and endeavour to surround them. And, in cases of intermittent fevers incredible numbers of *Spirillum* appear in the blood, and remain for several days “apparently without the slightest injury either to the circulation of the blood itself or to the heart and vessels,”<sup>1</sup> a fact which certainly supports my theory rather than that generally accepted. Again, it is well known that a blood clot, lying outside a vessel, causes an inflammatory exudation—that is, an accumulation of phagocytes—while a simple thrombus, which is in direct contact with the wall of a vessel, will cause no exudation; probably because there are sufficient numbers of phagocytes in the blood itself. From this point of view, the struggle between irritant bodies and white corpuscles, when it takes place directly in the vessels, may be spoken of as a kind of hæmitis.

<sup>1</sup> Colnheim, ‘Vorles. ueb. allgemeine Pathologic,’ 2 Auflage, Bd. 1, 1882, p. 475.

## The Structures connected with the Ovarian Ovum of Marsupialia and Monotremata.

By

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With Plate V.

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The Ovary of Marsupialia.—In the spring of 1883, I obtained an adult living female of *Phalangista* (*P. Vulpina*). Although I have only been able at present to work upon the ovaries of this Marsupial, I have no doubt that, in essential points—such as the structure of the ripe Graafian follicle—the present description will prove to be characteristic of the order. It is very difficult to imagine such important characters to be of less than ordinal value, especially when we consider that the structure, hitherto described as the normal mammalian Graafian follicle, appears to be practically constant in so many orders. I have not, therefore, hesitated to give the above title to this paper, although I hope to bring a posteriori proof when I can find time and material. There are also certain points in this ovary which I hope to further elucidate by more prolonged investigation.

The ovaries were hardened in chromic acid (of which the strength was increased during the process), and afterwards gradually in spirit. They were about 9 mm. in length, 6 mm. in width, and 7 mm. in thickness. Their surface was studded with large projecting follicles (see fig. 1  $\times$  9). In nearly all cases the sections were cut after staining the tissue as a whole,



and leaving it for a long time in melted paraffin. In this way it was possible to retain the entire contents of the follicles *in situ*. Borax carmine was chiefly used as the staining reagent. Fig. 1 gives a general view of all the structures seen under a low power in a complete section. The germinal epithelium is, as usual, a single layer of the very low columnar type. It is much lower than the cells lining the adjacent oviduct. The arrangement of the stroma in the thin "albuginea" seems to vary in different parts of the periphery of the organ, sometimes one layer being present, sometimes two or more, of which the cells cross one another. An irregular layer of small follicles (cortical layer of Schrön) is next met with, and there is a special tendency towards the formation of groups. Below this the follicles increase in size, but the largest project from the surface. In the centre of the organ there is a very richly-supplied "zona vasculosa," the vessels of which radiate outwards to the large follicles. In the rapid growth of a large follicle, some of the adjacent small ones are subject to such pressure that they become drawn out at right angles to the force, and parallel with the circumference of the large follicle (see fig. 4, *e.f.*). Some of the follicles distorted in this way are found considerably advanced in development, with the membrana granulosa many cells deep. I have no doubt that this explains many of the abnormally-shaped follicles described in the ovaries of higher mammals; it is certainly the explanation here.

The substance of the organ consists of a mixture of normal stroma cells and fibrous connective tissue. In the "tunica fibrosa" of the largest follicles the peripheral layers consist of fibrous connective tissue, the central of concentrically-arranged stroma cells, with abundant capillaries just outside the membrana granulosa (see fig. 6, *f.t.*). Yellowish granular cells, united into lobes and cords, are very abundant in the zona vasculosa (fig. 1), and isolated masses are also common, peripherally (fig. 4, *g.c.*). They are, doubtless, the traces of the Wolffian Body, which have been described in other mammals. There is no essential difference between the Marsupial ovary and that of higher mammals in any of the points described

above. But important differences exist between the Marsupial Graafian follicles and those hitherto considered universal among mammalia. Thus even in the lowly-magnified (fig. 1) the larger follicles are seen to possess a very thin and regular *membrana granulosa*, and the ovum in a (probably) full-sized follicle does not lie in a *cumulus proligerus*, but is embedded in a substance corresponding in position with the *liquor folliculi*. Minute examination shows further peculiarities. The young follicles appear to be normal; the ova are at first surrounded by a single layer of flattened cells—the follicular epithelium. These thicken and then multiply, forming a *membrana granulosa* several cells deep. It is then noticeable that the cells in immediate proximity to the ovum are not columnar, and thus the layer of radiating cells round the ovum, so characteristic of the higher mammalia, is entirely absent. In fact, these cells show a tendency towards the flattened shape, and columnar cells never appear in this position throughout the further stages of the follicle.

The *granulosa* cells have distinct readily-staining nuclei; they are very easily changed in shape, and it is hard to distinguish their outlines. They are probably polyhedral, with a frequent tendency to be drawn out parallel with the side of the ovum. The layer round the ovum sometimes shows a tendency to split away from the others. There is sometimes an indication of columnar structure in the cells near to the follicular wall. The great want of persistence in form shown by these cells is seen when the *granulosa* has split away from the follicular walls (as very frequently happens). Then the cells are drawn out into long threadlike processes, of which many are left behind, fixed to the *membrana propria*. The latter is extremely thin. Subsequent examination of a fresh specimen, and after the use of other reagents, confirms the above description. The outer *granulosa* cells are columnar, the others polyhedral. The outlines are much more distinct than in the figures which were drawn from the organ hardened in chromic acid.

The *zona pellucida* (?) is excessively thin throughout the

whole of ovarian development. In one instance I calculated that it was .0013 mm. in thickness, and, even allowing for a considerable error, the result is very remarkable. The ovum itself (as prepared by the method above described) appeared as a very delicate network, which did not stain with reagents. The nucleus (germinal vesicle) was enclosed in a distinct limiting membrane, and appeared in two forms distinguished by their behaviour with staining fluids. In the one case it stained deeply (see fig. 3), and in the other not at all (see fig. 2). It generally showed a coarse network, and it always contained a distinct spherical dark-coloured nucleolus (germinal spot). I could never distinguish the nucleus in the ovum of a large follicle. In the smallest ova the nucleus appeared to be always central, and it became eccentric during increase in size (compare figs. 4, *g*, *f*, with 2 and 3). The first traces of a cavity in the granulosa resemble the corresponding change in higher animals, and at first the clot formed in the cavity (by the reagents) appears to be identical with the coagulated liquor folliculi. The former shrinks away from the walls of the cavity, remaining attached by threads, and in structure it is homogenous or finely granular. While the clot is thus normal the ovum is still embedded in the thickened granulosa cells, which at this stage may be called a cumulus proligerus. At this point the only essential characteristic of the follicle is the absence of radiating columnar cells round the ovum (see fig. 4). During further development the follicle increases immensely in size, and the granulosa cells form the abundant follicular contents so rapidly that (as the follicle widens) the layer is much reduced in thickness. The cells round the ovum undergo a similar change, and thus the ovum comes to be isolated in the follicular contents, surrounded by vanishing layers of cells (see fig. 5). The change continues until in the large follicle the ovum is left embedded in the central substance, completely detached from the membrana granulosa, and only bounded by its zona pellucida (see fig. 6). Nevertheless, there are slight thickenings on the granulosa at the point nearest to the ovum, probably due to the fact that

the layer was thickest here, and has consequently here remained in greatest amount (fig. 6). In one case I observed one or two layers of cells still surrounding an isolated ovum in an apparently ripe follicle. While the changes in the granulosa have thus progressed beyond any stage met with in higher mammals, the central contents of the follicle have also undergone modification. This is seen in the fact that the ovum, although in the follicular contents, was always imbedded (in the cases that came under my observation) on the side of the follicle that projected from the surface of the ovary, and generally (but not invariably) was placed near to the point of greatest projection (fig. 6). Again, the position of the ovum always bore a definite relation to the thickenings of the granulosa. But the ovum could not have any such definite relations if it were floating freely in a fluid such as the liquor folliculi. Therefore, it seems clear that the central contents of the follicle have become so gelatinous or viscous that the ovum can be imbedded without change of position (unless, indeed, it is anchored to the side, and of this I could find no proof). At the same time, the microscopic appearance of the central substance has entirely changed, assuming, finally, the appearance of a coarsely-granular network, which does not stain (see figs. 6 and 7). This network has not shrunk away from the granulosa during hardening, but when any contraction takes place, this generally produces a space between the granulosa and the follicular walls (see fig. 1), and the former often becomes much folded. In one instance only I found a considerable cavity between the central contents and the granulosa. Of course it is impossible to decide as to the condition of this remarkable structure during life without the investigation of the fresh organ. It may be that some of the appearances (e.g. granules) are due to coagulation produced by the reagents, but it is certain, from the above considerations, that a great change has taken place in the contents, and that ultimately there appears a substance very different from the ordinary liquor folliculi. In fact, nothing could be more different than the appearance of ordinary liquor from that of the central contents in question, when



both have been treated with the same reagents. And there is an almost equal difference between the appearance of the contents of a young and of an old follicle in the same Marsupial ovary (compare figs. 4 and 6). In the younger stages, when the follicular contents are finely granular, and the ovum is not yet isolated, the granulosa cells next to the central contents frequently appear swollen and vesicular, unstained, and with no distinct nuclei (fig. 4). Later there appears a distinct intermediate layer between the central contents and the granulosa. This layer is a network which seems to be continuous with the granulosa cells peripherally, and the strands of the contents centrally (see fig. 7). The intermediate network stains, and nuclei are common in it, apparently placed at the nodal points, so that it probably represents the granulosa cells, becoming stellate and arranged as a network, which again is continuous into the central contents. The latter generally show some slight distinction between the peripheral layer close to the intermediate network and the central part. Since writing the above, I have examined a fresh specimen, but unfortunately there were very few large follicles present. There was certainly some fluid present in the largest follicles, but the follicular contents could be forced out entire, and apparently surrounded by a wall, which may be the granulosa. I cannot be sure that this condition persists in the ripe follicle. The fluid is, as usual, albuminous. I could not detect the granules covering a network in the fresh state, but this may be due to the fact that the follicles were not ripe. I hope to work at the subject again.

The largest follicles seem to be about 2 mm. in their largest diameter, but there is not much difference between the long and short axes. In one follicle of about this size, the external fibrous part of the tunica fibrosa had thinned away at the most projecting pole (beneath which was the ovum), and it is therefore likely that the follicle was ripe. It is impossible to do more than speculate as to whether any of the gelatinous ovarian covering of the ovum (in the follicle) clings to it as an accessory layer after being received into the oviduct. The

position of the ovum towards the projecting side is against such a theory. The corpora lutea do not seem to be peculiar. The sizes of all structures mentioned can be readily calculated from the figures. Thus there are important distinctions between the Graafian follicles of a Marsupial and those of the higher Mammalia, and yet these distinctions are by no means in the direction of greater simplicity in the former, but rather the reverse. We shall find a very different state of things in Monotremes.

The Ovary of Monotremata.—Finding such interesting peculiarities in the ovary of Marsupials, I determined to investigate the same organ in Monotremes. Professor Moseley very kindly allowed me to take the ovary from a female specimen of *Ornithorhynchus*, which had been kept in spirits for many years in the University Museum, and also to look over and take what I could from some specimens of *Echidna* which had been similarly kept, but were not in a favorable condition for microscopic investigation. The ovary of *Ornithorhynchus* was flat (it may have been accidentally compressed) and oval, about 13 mm. long, 7 mm. wide, and 2 mm. thick. The edges were sharp. It was impossible to make out the shape or size of this organ in *Echidna*. In both cases I treated the organ as previously described after gradual hardening in spirit. Fig. 8  $\times$  9 gives the appearance of a transverse section across the ovary of *Ornithorhynchus*. The follicles are confined to the edge of the section, and therefore the surface of the organ. There does not appear to be any distinct arrangement of follicles according to their size, but the small ones always seem to be very near the surface. There were indications that the large follicles are constricted off, in the presence of a deep furrow encircling some of them. It is probable that this would be far more distinct in the fresh organ. Such constriction is a much more important character than mere projection, which occurs in Marsupials, and in some higher orders. There was, of course, no trace of the germinal epithelium, and nothing could be ascertained as to the true nature of the stroma. There certainly is

a layer, condensed from the general substance of the organ, upon the outside of large follicles, but it was impossible to investigate its minute structure. Blood-vessels were not common or large in the central tissue of my sections (taken transversely across the middle of the organ). It is certain that they were much contracted, and perhaps often obliterated. But while I was able to make very little out of the general structure of the organ, I was more fortunate with the follicles, in which some most important points were still recognisable. On first examining some of the largest follicles (about 1 mm. in diameter, but probably contracted), I found the ovum much shrunk, and generally disintegrated, but between it and the follicular walls there were a number of short segments of the same curvature as the follicle (or rather less). Further examination showed that these segments consisted of two layers of about equal thickness, the inner being homogeneous, and the outer evidently composed of a single layer of cutical cells. Careful search over a large number of sections enabled me to prove that the outer layer was the follicular epithelium—a single layer during the whole time that the ovum remains in the follicle. Therefore *Ornithorhynchus* follows the lower type, and never gains the characteristic mammalian granulosa with many layers of cells. In many follicles of the largest size I found the single-layered epithelium still adherent to the wall over a large part of the circumference. The epithelium formed a very persistent layer, and in many cases it remained continuous when separated from the follicle by the contracting ovum. When thus separated, it was possible to observe the layer from the surface, and it then appeared as a mosaic of cells. The inner part of the segments is an investment of the ovum—probably the zona pellucida. This layer is generally closely adherent to the epithelium, and thus both were shattered when the ovum contracted. It is very common to find a third finely-granular layer of about the same thickness as the others on the inside of the segments. Those few fortunate sections which showed these layers in situ proved that this third layer is the external part of the ovum (see fig. 12). Under high

powers it is possible to make out a fine layer between the zona and the third layer, and one (or sometimes two) fine lines in the inner part of the zona, parallel with its surface. The latter may be an indication of a laminated structure, or it may be an optical effect, or caused by changes in the tissues. The minute structure of these layers must be investigated with better material. The result of these facts is that the ova of Monotremes practically fill their follicles, and are of considerable size. In this specimen the ova had often shrunk to half the diameter of the follicle, and the intervening space was occupied by the loosely-packed debris of the epithelium and zona. High powers showed that the follicular epithelium rests on a thin but distinct membrana propria, which it often draws with it away from the follicular walls. In young follicles the epithelium is thin, but it soon becomes cubical, and the zona appears, at first staining slightly, and of less thickness than later; always apparently homogeneous. Concerning the ova, it is an obvious suggestion that in such large structures there must be a well-marked distinction into food-yolk and germinal-yolk. It is also probable that segmentation is unequal, perhaps partial. The large ova were generally disintegrated, but from occasional specimens I found that the nucleus (germinal vesicle) was eccentric, having been central in small ova. The nucleus had a distinct limiting membrane, within which it had shrunk (see fig. 11). There was a well-marked nucleolus (germinal spot). Within the external, finely-granular, deeply-staining layer of the ovum; there was another irregular layer of finely-granular material which did not stain deeply, within which there were a number of various-sized spheres and spheroids, some of them very large. These were finely granular under high powers, and did not stain deeply. Again, in the centre, there were traces of material similar to that just external to the spheres (and which seemed to pass between them). This may be the result of change, but I do not think that it is so entirely. Here, again, confirmation must come with better material. It seems probable that the darkly-staining, peripheral, vitelline layer is the most recently-formed



secondary yolk. These finely-granular layers, and the spheroids within, together with the external investments, have a very obvious resemblance to Waldeyer's figure of the follicle of a fowl (fig. 194, p. 184, vol. ii. of the English translation of Stricker). In fig. 11, the nucleus is situated in the layer of spheroids; it is probably on its way towards the circumference.

The corpora lutea are very remarkable. The most important change that takes place in their formation seems to be the thickening of the tunica fibrosa, which thickening continues until the cavity is almost, or quite, obliterated. This change appears to begin early, and sometimes to be the only change, but of this I cannot speak with certainty. In the immensely-thickened layer blood-vessels are common. Such structures, with walls of all degrees of thickness, were very common indeed (see fig. 1). Sometimes the lumen appeared empty, and sometimes it appeared to contain a shrunken basement-membrane with a few cells, perhaps of the follicular epithelium. In other and far rarer instances I observed masses of yellow cells within the thickened walls. It may be that this last is the initial, the former the ultimate stage, and that thus the corpora lutea are not very remarkable in this animal; but the objection to this is, that the masses of yellow cells are comparatively rare, and that they do not form large and important masses, and further, that the former changes seem to be taking place at an early date, as far as one can judge. In a few cases the lumen contained a yellow shrunken clot which had also stained the adjacent tissues. This whole question needs further elucidation.

The condition of the ovaries of *Echidna* was such that I could make out nothing, except the important fact that the follicular epithelium and zona pellucida are similar to *Ornithorhynchus*. I was enabled to ascertain this from an examination of scattered segments, such as I have described.

These results as to the relation of the ovum within the ovary are very important, being characters that persist over important sections of the animal kingdom. If the conclusion that I have

expressed above concerning the follicular epithelium of Monotremes be correct (and of this point I am sure), it will, perhaps, more than any other character, serve to place this order on a totally different level from the other mammalia. It sinks the differences between Marsupialia and Monodelphia in an equal divergence from both. The result is, I think, astonishing; the gap is almost greater than could be expected—not only in the Monotreme's downward affinities, but in the upward affinities of the Marsupials. In other points ("The Tongue of Ornithorhynchus" in this Journal for July, 1883) I have shown indications of transition between the two orders, but there is no such tendency here, where the characters are probably of very deep significance.

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## On the Skeleto-trophic Tissues and Coxal Glands of *Limulus*, *Scorpio*, and *Mygale*.

By

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With Plates VI, VII, VIII, IX, X, XI.

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1. Introduction.
2. The entosternites and massive connective tissue of *Limulus*, *Scorpio*, *Mygale*, and *Apus*, and the colloid tissue of *Mygale*.
3. The lacunar connective tissue of *Limulus* and *Scorpio*: the pigmentiferous tissue and finer blood-vessels (arterioles).
4. Reticular connective tissue of *Limulus*.
5. The membranous connective tissue of *Limulus* and *Scorpio*.
6. The large-celled (embryonic) connective tissue of *Limulus* and *Scorpio*.
7. The cartilage-like (capsuligenous) connective tissue of *Limulus*.
8. The blood-corpuscles of *Limulus* and *Scorpio*.
9. The coxal glands of the Arachnida.
10. Minute structure of the coxal glands of *Scorpio*.
11. Minute structure of the coxal glands of *Limulus*.
12. Minute structure of the coxal glands of *Mygale*.
13. General conclusions as to the coxal glands.

THE inquiry into the relationship of *Limulus* to the other Arachnida with which I have now for some time occupied myself, has led me to institute a detailed comparison of the minute structure of the tissues of these animals. Many of my observations have been made by means of sections of whole animals, or of special organs—others have been made upon the fresh and living tissues of *Limulus*, of *Scorpio*, and of *Mygale*, of all of which I have been able to obtain live examples for study in my laboratory in London.

In previous publications<sup>1</sup> I have given some account of the remarkable internal skeleton or entosternite of *Limulus* and *Scorpio*. I have also drawn attention to the coxal glands of the Arachnida, previously unknown, and pointed out their agreement with the "brick-red glands" of *Limulus*.<sup>2</sup> Hitherto I have not published any account of the microscopic structure of the entosternite, nor any sufficient explanation of the microscopic structure of the coxal glands. I propose to supply these requirements in the present memoir, and, inasmuch as the entosternites of *Limulus* and the other Arachnida are histologically a variety of the connective tissue, or vaso-fibrous tissue, it will be convenient to include here a description of the more important varieties of tissue belonging to the skeleto-trophic<sup>3</sup> group, which present themselves to our notice in *Limulus* and its allies.

It will be found that there are remarkable points of agreement between *Limulus* and *Scorpio* in respect of some of these tissues. On the other hand, the observations have value on account of the necessity which exists for a detailed and comprehensive study of the connective tissues and other tissues of the skeleto-trophic group in both Arthropoda and Mollusca, before we can pretend to offer any satisfactory account of the vascular system in those groups, and of the "lacunar" connection between arteries and veins, which is confidently described and discussed by all zoologists, but has never yet been demonstrated to exist, in a manner satisfying the requirements of modern histology.

With regard to the anatomical connections of the entosternites in *Limulus* and *Scorpio*, and especially with regard to the complicated series of muscles which are attached to these structures in each of those animals, I would refer the reader to a memoir now in course of publication by the Zoological Society

<sup>1</sup> "*Limulus* an Arachnid," this Journal, 1881.

<sup>2</sup> "*The Coxal Glands of Scorpio*," 'Proc. Royal Soc. London,' No. 221, 1882.

<sup>3</sup> I regard the skeletal, vascular, and hæmolymph derivatives of the mesoblast (including Mecznikow's "phagocytes") as forming a natural group of tissues to which the name "skeleto-trophic," is appropriate.



of London, where I have described and figured (with the assistance of my pupils, Mr. Benham and Miss Beck) the entire muscular and skeletal system of *Limulus* and *Scorpio*.

The observations recorded in the present memoir, in reference to the structure of the coxal glands, will be found to have importance, not only as adding one more to the many proofs of the necessity for referring *Limulus* to the class *Arachnida*, but also on account of the minute structure of the special cells of the glands, which has a physiological significance.

## 2. THE ENTOSTERNITES AND MASSIVE CONNECTIVE TISSUE OF *LIMULUS*, *SCORPIO*, *MYGALE*, AND *APUS*.

I have already figured in this Journal the entosternites of *Limulus* and *Mygale*, and imperfectly that of *Scorpio*. I am now able to submit to the reader more complete drawings<sup>1</sup> of that of *Scorpio* (Pl. VI, figs. 5, 6, 7), and have introduced into the same plate those of *Limulus* and *Mygale* for comparison.

The entosternite of the *Arachnida* (including *Limulus*) is essentially a plate of massive skeletal tissue placed in the median line of the prosoma, and provided with various paired tendon-like outgrowths, to which muscles are attached connected with the body wall and with the limbs. The plate itself is moveable, having only delicate membranous connections of skeletal tissue with the chitinous sclerites of the tegumentary system, in addition to its muscular attachments. The bulk of its substance is developed in a plane between the ventral nerve-cords and the alimentary tract. In *Limulus* and in *Mygale* the entosternite does not embrace the alimentary canal nor the nerve-cords in its substance, being simply continuous with the delicate connective tissue which surrounds those organs; whereas in *Scorpio* the thickening and specialising of the connective tissue extends from the main plate, so as to form a subneural arch below the nerve-cords (Pl. VI, fig. 6, *snp.*), thus enclosing the nerve-cords in a neural canal (N C), whilst simi-

<sup>1</sup> For these I am indebted to Miss Beck. They were prepared from dissections of a number of specimens of *Buthus cyaneus*, of Ceylon.

larly on its dorsal surface the alimentary tract is enclosed in a gastric canal (G C, in Pl. VI, fig. 5), and the dorsal artery or aorta in an arterial canal (A C). In the Scorpions, also, the connective tissue continued from the sides of the median plate is tough and dense, so as to form an expansion of the entosternite in the form of a right and left "posterior flap" (*pf*, in Plate VI, figs. 5, 6), by which the entosternite is brought into continuity with the lateral walls of the body. Thus, in the Scorpions the entosternite assumes the form of an obliquely-placed "diaphragm"—a name applied to it by Newport—cutting off the cavity of the prosoma almost completely from the cavity of the mesosoma. This lateral extension of the entosternite does not occur in either *Limulus* or *Mygale*.

The various processes and ridges of the entosternites of *Limulus* and of *Scorpio* are named in the description of Pl. VI. In attempting to determine the equivalent structures in the entosternites of these two animals it is necessary to make use of the indications afforded by the attachment of the muscles. This is a subject which I do not propose to treat here, since I have dealt with it in the paper already mentioned in the 'Transactions of the Zoological Society.' I may, however, briefly point out that only that small portion of the Scorpion's entosternite which immediately roofs in the neural canal appears to be equivalent to the "body" of the entosternite of *Limulus*. The right and left anterior processes (RAP, LAP, in the figures) appear to be equivalent in the two animals, whilst the two pairs of lateral rod-like tendons in the *Limulus* entosternite (ALR, PLR) are represented by the latero-median processes (*lm p*) of the Scorpion. There is nothing in *Limulus* corresponding to the subneural arch (*snp*) of *Scorpio*, nor to its anterior inferior pair of delicate tendons (*asp*).

It is interesting to find in *Mygale* a much closer agreement with *Limulus* as to the form of the entosternite than we can detect in *Scorpio*. The two pairs of lateral rod-like tendons are represented by three similar pairs in *Mygale* (Pl. VI, fig. 4); the bifurcated posterior median process is present in both, and the general form of the "body" with its neural fossa (*nr*) and

lateral processes is identical; only in *Mygale* we find a fuller development, a repetition of well-marked processes corresponding to successive segments of the body, which is not so well-marked in *Limulus*. In *Mygale*, as in *Limulus*, there is no subneural arch, nor is the gut nor the aorta involved in any dorsal excrescence of the tendinous mass.

An entosternite like that of *Mygale* exists in the common British *Epeira diadema*.

The tissue of which this "floating" skeletal plate is formed has a very closely similar structure in all three genera. It is represented in Pl. VII, where figs. 1 and 2 from *Scorpio*, fig. 3 from *Limulus*, and fig. 4 from *Mygale* are all drawn to the same scale.

**Histology of the Entosternite of *Limulus*.**—The large size of the entosternite of the King Crab renders an examination of the character of its tissue more satisfactory than that of other Arachnida. When cut through with a knife, the substance has much the appearance of Vertebrate hyaline cartilage; it is of the same consistence and translucence, and is, in fact, superficially so much like Vertebrate cartilage that one is led to expect the presence of the same chemical bodies in its composition. This expectation is, however, fallacious. My colleague, Prof. Schäfer, F.R.S., has been kind enough to make a chemical examination of this tissue, and he reports that it does not yield chondrin nor gelatin, but nearly equal quantities of chitin and of mucin, with a very small quantity of albuminate.<sup>1</sup>

<sup>1</sup> Professor Schäfer has furnished me with the following additional note:—"The reasons I have for thinking that the substance you placed in my hands may probably contain 'chitin' are chiefly negative. After removal of the mucin the residue is insoluble in almost everything I have tried except concentrated sulphuric acid, and in this it does not dissolve at all readily without the aid of heat. It swells in alkalies, apparently without becoming dissolved, even on heating. The solution in concentrated sulphuric acid, dropped into boiling water and kept boiling a short time, yields a substance in solution which dissolves cupric hydrate in presence of caustic potash, but on boiling the mixture I was not able to obtain much evidence of the formation of cuprous oxide, although I once or twice have thought I could detect traces.

The presence of chitin in a tissue belonging to the skeletotrophic group, and derived from mesoblast, is a novelty. It appears to have been too readily assumed that the connective tissues of Invertebrata correspond in their chemical nature with those of Vertebrata, and the notion that chitin is a product confined to the activity of the tissues of the epiblast has been hitherto adopted without a sufficient basis in fact. The skeletal product of the protoplasmic cells which build up the entosternite of *Limulus* is chiefly chitin, and I am led, from the behaviour of the fibres and trabeculæ of the connective tissue in other regions of the body of *Limulus*, and in other Arthropoda, to suspect that this substance takes the place of collagen and chondrin in the skeletal tissues of Arthropoda. The differences between elastin (the formed substance of Vertebrate elastic tissue) and chitin are so slight that one would not be greatly surprised at the presence of the latter body as a main constituent of connective tissue in some groups of animals were it not for the fact that an attempt has been made by so eminent a physiologist as Kühne to definitely distinguish between the chemical possibilities of epiblast and mesoblast in regard to this very substance.

The presence of chitin in the entosternite of *Limulus* once and for all establishes the fact that this body can be produced as a main constituent of the tissues of the mesoblast, just as characteristically as it is by the epiblast.

The microscopic structure of the entosternite of *Limulus* is essentially as follows:—a firm, homogeneous, or sparsely fibrillated matrix in which are embedded nucleated cells, generally

The formation of grape sugar from chitin under the above treatment with sulphuric acid is characteristic, but the evidence of its presence I could get with the small amount of dry material available was but feeble, and I am not prepared to say positively that the substance is 'chitin,' or even that it is chiefly chitin; but considering its solubilities, or rather insolubilities, it is probably either that substance or a mixture of that and a substance allied to keratin. There is also the possibility to be taken into account that it is a material hitherto undescribed of the same nature as chitin, but differing from it in certain minor particulars: it would, however, be necessary to get it very pure and in some quantity to make the investigation of any value."



arranged in rows of three, six, or even eight, parallel with the adjacent lines of fibrillation. Some of the cells are isolated, or in pairs, whilst in the deeper parts of the tissue, in place of rows of such cells, we find irregular clusters, or groups of cells (four to eight). The relative abundance of matrix and cells is shown in Pl. VII, fig. 3, which represents a piece of the tissue where the fibrillation is not very strongly marked, and not all in one plane.

The nuclei of the cells stain deeply when a piece of the tissue is removed from alcohol and placed in borax carmine ; at the same time the matrix also is strongly, but not so deeply, stained, whilst the protoplasm surrounding the nuclei does not stain at all.

Owing to the fact that the entosternite is a sort of central tendon in which the tendons of several muscles converge, it is possible to obtain sections of different character from different parts of it. By cutting the divergent processes parallel with their long axes, we can obtain sections showing strong fibrillation of the matrix parallel with the long axis of the process, and with rows of cells all strictly parallel to the same lines (Pl. IX, fig. 2). Such sections most closely resemble Vertebrate tendons, such as those of the rat's tail, but the cells do not appear to be flattened, as in Vertebrate tendon, and the matrix is not fissured so as to form distinct bands, but rather indicates a tendency to such fissuring in the fact of its fibrillation. A comparison with white fibrous cartilage of mammalia is, perhaps, more nearly justified.

Again, by taking sections more deeply, we may obtain pieces of tissue in which the lines of fibrillation from various processes of the entosternite are converging and crossing one another. Very complicated grouping of cells and fibres results from this convergence.

Lastly, in the more central parts of the entosternite we find the matrix no longer showing a tendency to fissuring so as to suggest the descriptive term "fibrillated," but we find actual branching fibres developed in the hyaline matrix, the branching fibres being of denser substance than the rest of the matrix

and enclosing in their reticulations groups of cells embedded in matrix (Pl. IX, fig. 1). This structure very closely resembles that of Vertebrate elastic fibro-cartilage.

The chief difference between this tissue and typical Vertebrate cartilage, so far as form is concerned (apart from chemical constitution) is in the mode of multiplication of the embedded cells. The cells are, it is true, just as in Vertebrate typical cartilage, embedded in a firm structureless matrix, which fits closely to the periphery of the cell protoplasm, being only distinguishable therefrom by the slight difference of the refractive indices of the two substances. But it is characteristic of the present tissue for its cells to multiply linearly, that is by growth along and division across one axis: rarely only do they multiply along an axis at right angles to this. The tissue is in fact essentially fibrous (monaxial cell division) or rarely membranous (biaxial). At the same time the cells resulting from the division of parent cells remain in closely adherent groups (either rows or irregular masses) as shown in Pl. VII., and only here and there do we find isolated cells entirely surrounded by matrix. This implies that the activity of such cells in producing matrix around themselves is not great, or at any rate, not uniformly present. In contrast to this we find that a cell of Vertebrate cartilage no sooner takes its origin by division from a parent cell than it becomes separated from that parent by a layer of matrix (capsule) which rapidly increases in volume. Every cell appears to be thus constantly active in developing matrix and becomes separated from its parent cell by a considerable interval. At the same time all the cells of a tract of Vertebrate cartilage are approximately equally active in division, and all may be rapidly growing and multiplying by division or all may be comparatively quiescent. This equality and isolation of the cells of typical Vertebrate cartilage is accompanied by another feature in growth in which it differs I think constantly from all but the deepest parts of the tissue which forms the tendinous entosternite of *Limulus*. Vertebrate cartilage (excepting the white fibro-cartilage which is transitional in its characters between tendon and typical

cartilage) is not fibrillar (monaxial) nor membranous (biaxial). It is block-like, and its cells multiply equally in the three directions corresponding to the axes of a cube (triaxial cell-division). It is not developed under conditions of pressure or tension along constant lines or planes but is subject to a uniform tension or pressure on all sides. This is true even of elastic or reticular cartilage.

It is extremely difficult to decide whether the word "cartilage" can be properly applied to the entosternite of *Limulus*. If "cartilage" be defined chemically it certainly is not appropriate. But is it desirable to define the species of a histological system with reference to chemical characteristics? How could we thus define "epidermis?" Should we not thus be led to separate too widely the chemically-differing gland epithelia? On the other hand, it is not clear that the word "cartilage" has been used by either anatomists or histologists with any sufficient morphological limitation. So far as mere texture and quality of substance goes, the entosternite of *Limulus* would be spoken of as cartilaginous. It seems, however, possible to consider the species "cartilage" as morphologically defined by the isolation of each one of its constituent cells in a firm matrix, and by the triaxial multiplication of those cells, whether the matrix be homogeneous or fibrillated (fissured), or penetrated by reticular condensations. In this sense the entosternite of *Limulus* cannot be said to be "a cartilage," nor, indeed, can the white fibro-cartilage of Vertebrates be properly so called.

For the present, then, it will be best to speak of the tissue which forms the entosternite of *Limulus* as a chitinigerous fibromassive skeletal tissue. The terminology of histology has not really at present a proper name for it, and it would take us too far out of our way on the present occasion to devise a system of nomenclature of skeletal tissues in which this particular variety should find a properly-defined place.

In *Limulus* this same tissue is found as the constituent of six small entochondrites, which exist in the successive segments of the mesosoma, placed in the middle line ventrad of the nerve-cords, and giving attachment to muscles. It is also

found forming the cortical or outer layer of the entapophysial ligament—a strong band which connects the successive dorsal entapophyses with one another. It also, in a modified form, constitutes the tendons of the branchio-thoracic muscles (Pl. IX, fig. 4.) For figures and an account of the relations of these parts I must refer the reader to my paper above mentioned, on the muscular and skeletal system of *Limulus* and *Scorpio*.

**Histology of the Entosternite of the Scorpion.**—The entosternite of the Scorpion is composed of a tissue very closely similar to that just described. The cells are somewhat smaller in size, and more constantly disposed in elongated groups, two—eight or more placed in a row—in what may be called “fissures” of the dense but slightly fibrillated matrix. As in the case of the *Limulus* entosternite, so here the tissue appears to yield no gelatin nor chondrin, but chitin and mucin. The staining with Borax carmine is taken up in the same way as in *Limulus*, viz. hardly at all by the cell protoplasm but strongly by the matrix and the nuclei.

In Pl. VII, fig. 1, a piece of the entosternite of *Androctonus funestus*, Ehr., is drawn, showing, as well as the characteristic tissue, the insertion of muscular fibres into it by means of the continuity of the inter-muscular connective tissue with the tissue of the entosternite. In the upper part of the figure is also shown the continuity of the fibroid tissue of the entosternite with the characteristic “lacunar connective tissue,” which forms so large a part of the packing of the viscera in *Scorpio* and *Limulus*, and is especially richly developed between the cæca of the great gastric gland or so-called “liver” (see below).

In *Scorpio* I have found the fibroid tissue of the entosternite developed only in one other region, namely, in a small tendinous plate overlying the base of the pectines—modified appendages corresponding to the first pair of branchial appendages of *Limulus*. This plate corresponds in position and muscular connection, as in structure, with one of the small entosternites of the mesosoma of *Limulus*.

**Mygale.**—The fibro-massive tissue of the entosternite of



Mygale is more strongly fissured than is that of either *Limulus* or *Scorpio*, and concurrently we find larger groups of cells gathered together than in the two other forms (Pl. VII, fig. 4). Cell-protoplasm, nuclei and matrix present the same appearance and the same staining reaction as in *Limulus* and *Scorpions*.

Over and above this the peripheral regions of the entosternite of *Mygale* present a feature which I have not observed in either *Limulus* or *Scorpio*, and which is not shown in its more central region, from which the specimen given in Pl. VII, fig. 4, is taken. In a large fissure here and there one observes in the peripheral outgrowths of the entosternite, besides numerous nucleated cells, a highly refringent substance of a very pale brownish tint, quite homogeneous and transparent, except for the fact that it is arranged in spheroidal masses more or less closely pressed against one another, the space left between such spheroids and the wall of the fissure being occupied by the cells elsewhere characteristic of the fibro-massive tissue. I do not know the chemical nature of this substance; its relation to solvents shows that it is not fat, and by its homogeneity and refringency and colour it differs widely from the matrix of the fibro-massive tissue. It may be spoken of as "colloid substance" (see Pl. XII, fig. 3, *col*). Its presence in the nests of cells scattered through the fibroid matrix is clearly due to the activity of those cells, and may be compared to the presence of fat in cartilage cells. But it is to be noted that this colloid substance is not like fat, an entoplasmic product; it does not lie within the protoplasm of the cells, but is ectoplasmic, that is, around and outside of them, just as is the common fibroid matrix, in the formation of which some of these same cells must be active.

The production of this colloid may, perhaps, be regarded as a functional degeneration or change of chemical activity of the outlying regions of the fibro-massive tissue of the entosternite, in which alone it occurs. It occurs, however, in another organ in a very marked form without any relation to fibroid skeletal tissue—namely as a sort of skeleton or framework to the

trabeculæ of the coxal gland (Pl. XII, fig. 1, *col*). Its place in *Scorpio* and in *Limulus* in relation to the coxal gland is taken by another variety of connective tissue. It seems that in the coxal glands of *Mygale* the colloid substance with cells scattered in it and widely separated from one another is entitled to be regarded as a definite tissue, for which the name of "dense colloid tissue" may temporarily serve. It has a superficial resemblance to the substance of foetal spongy bone formed during ossification in cartilage.

*Apus*.—Though the great development of the entosternite is characteristic of the Arachnida, and is one of the common features of structure which, as Straus Durkheim pointed out, necessitates the placing of *Limulus* in the class Arachnida, I have yet within the past few months discovered that structures identical in form, position, and histological composition with the Arachnidan entosternite exist in the Crustacea.

The only well-marked separable entosternite which I have at present found among Crustacea is in *Apus cancriformis* and *Apus productus*. The plate lies dorsal to the nerve-cord below the alimentary tract in the mid line of the body between the two great mandibles, some of the muscles of which are inserted into it. It appears also to receive a muscle on each side from the maxillæ, and possibly some from the ventral body wall. It is shown in Pl. VIII, figs. 2 and 3, as separated from its muscular attachments. A section showing the minute structure drawn to the same scale as the sections of entosternites on Plate VII is represented in Pl. VIII, fig. 1. It will be admitted at once that we have here a fibroid skeletal tissue of exactly the same character as that presented by the Arachnidan entosternite.

Naturally one expects the tendinous rods and processes within a Crustacean thorax to be inversions of the epidermal cuticular system. That this entosternite of *Apus* is not so is proved, firstly, by its want of any direct continuity with the integument as determined by means of a series of transverse sections of *Apus*, and secondly by its histological character. The apodemes and chitinous ingrowths of the integument

common in all larger Arthropoda have a totally different and thoroughly characteristic structure.

Having found this small but undeniable true internal skeleton in one Crustacean, I have been anxious to look for it in others. At present I have only examined hastily some Decapoda, and I find that there (*Palæmon*, *Astacus*) the incomplete archway formed by the thoracic epidermal apodemata is completed anteriorly in the median line by a small piece of fibro-skeletal tissue similar in histological character to that forming the entosternite of Arachnida. It is, however, extremely small, and obviously the development of floating skeletal plates formed of dense connective tissue does not take a prominent place in the economy of the higher Crustacea as it does in that of the Arachnida. It is remarkable that the most archaic of living Crustacea (*Apus*) should possess a well-marked though small entosternite of mesoblastic origin.

Probably enough such entosternites of dense connective tissue will be found, when properly looked for, in not a few other Arthropoda.

N.B.—In all the above cases the tissue of the entosternite is non-vascular.

### 3. THE LACUNAR CONNECTIVE TISSUE OF *LIMULUS* AND *SCORPIO*; PIGMENTIFEROUS TISSUE AND THE FINER BLOOD-VESSELS.

The most abundant and widely-distributed variety of connective tissue in *Limulus* and the Scorpions is that which I propose to term "lacunar connective tissue." This tissue "packs" the cæca of the gastric gland and genital ducts, and is also found between the bundles of muscular tissue. It passes over at certain points into membranous connective tissue, and again in the neighbourhood of the entosternite is suddenly transformed into the fibro-massive skeletal tissue of that organ.

The lacunar connective has a very definite character which is shown in Plate X, figs. 1 and 2, and in Plate XI, figs. 1, 2, 3. The distinctive features about this tissue as compared with the fibro-massive skeletal tissue which we have noticed above is (1) the very small amount of skeletal matrix and the relative

large proportion of the protoplasmic cellular element; (2) the disposition of the cells around spaces or lacunæ which are of an oval or polygonal character, and communicate freely with one another. We may conceive of the conversion of the fibroid skeletal tissue into lacunar tissue by supposing the fissures in the skeletal substance in which the cells are placed to become dilated and widened at the expense of the skeletal substance itself, which would be so reduced by this process as to become a mere membrane separating the various fissures from one another, and even breaking down altogether at numerous points so as to allow one enlarged fissure or lacuna to communicate with another. Then further we should make the cells adhere closely to the wall of the enlarged space in which they exist, projecting irregularly in an amœboid fashion into that space, their protoplasm being more abundant than was the case in the confined cell-groups of the fibro-massive skeletal tissue.

In *Limulus* the cells of the lacunar tissue lying between the cæca of the gastric gland contain usually each a single spherical drop of a highly refringent substance of a yellowish brown colour, which is of a fatty nature, though not readily soluble (Pl. XI, fig. 1 *d*). In *Scorpio* numerous smaller granules are scattered in the protoplasm of the corresponding cells, resembling the granules which occur also in the blood-corpuscles (Pl. XI, fig. 3 *d*).

The formation of the lacunar tissue is so regular as to give the appearance in section of a number of polygonal areolæ fitted side by side, as though the section had traversed a mass of closely packed capsules. We may therefore apply the term 'capsule' to the skeletal substance (*b* in the figures) which forms the firm boundary of each of the polygonal spaces whilst the term 'lacuna' applies to the space (*e* in the figures) left centrally between the cells lining the capsule.

The membranous skeletal substance which forms the capsules appears to be chitinous in nature like the matrix of the fibroid tissue of the entosternite. After maceration it may be obtained (in the case of *Limulus*) free from the protoplasmic



cells and when seen in thickish sections has the appearance shown in Pl. XI, fig. 8.

The lacuna or central space of each capsule (always communicating by a gap in its wall with the lacuna of one or more neighbouring capsules) contains an albuminous liquid, which sometimes is seen in the form of a coagulum in sections of the tissue hardened in spirit. According to varying conditions (probably the state of nutrition of the animal) the lacunæ will be large, and the protoplasm of the cells lining the capsule sparse and thin, spreading as a film over the surface of the capsule, or on the other hand, the lacuna will be much reduced, possibly obliterated by the rich development of the protoplasm of the lining cells, which then stand out as large masses, pressing against one another and filling up the lumen of the capsule. These two states of the lacunar tissue are probably also permanently exhibited in varieties of the tissue from different parts of the body.

The exceedingly important question now arises—‘What is the nature of the lacunæ and their contained fluid?’ At first sight one is disposed to take the view that these lacunæ are part of the general “lacunar blood system” universally ascribed to the Arthropoda, and very possibly they are so. But at present I have no evidence to show that they are. I have never found blood-corpuscles floating in these lacunæ, nor have I seen any blood-vessel enter into open communication with them. At present, also, I have not succeeded in injecting them with soluble Berlin blue when such injection is made either by way of the arteries from the heart, or by way of the great venous sinuses by use of a puncturing syringe. The further investigation of the relation of this tissue to the vascular system will occupy some time and can be most appropriately discussed in connection with an examination of the circulatory and respiratory organs with which I am occupied.

The lacunar connective tissue of both *Limulus* and *Scorpio*, unlike the fibro-massive skeletal tissue, is vascular. Not unfrequently one observes in a section of this tissue fine blood-

vessels lying in the capsular wall of contiguous lacunæ. Such fine vessels are seen in Pl. XI, figs. 2 and 3 *bv*.

These vessels contain when thus observed in sections, a blood clot and blood corpuscles. Their proper walls appear to be formed by a single layer of cells. I have not obtained any evidence as to the termination of these vessels. On the one hand it is possible that they terminate in cords of cells set in single file which become excavated so as to form true capillaries; on the other hand they may open into the lacunæ. The latter is by far the more likely mode of termination, but at present I have not succeeded in observing it.

Besides blood-vessels we find in *Limulus* other fine spaces excavated in the capsular walls of the lacunar tissue. These are the genital ducts and testicular ampullæ of the male. The female genital canals of *Limulus* and both the male and female glands of *Scorpio* are of larger size so that they are simply packed by the lacunar tissue just as the gastric cœca are. But the cœca and finest canals of the male apparatus of *Limulus* are so small and so much ramified as to become very intimately interwoven with the lacunar tissue (see Pl. X, figs. 1 and 2 *c*).

Pigmentiferous tissue is largely developed in *Limulus*, and to a less extent in *Scorpio*, by the simple accumulation of pigment granules in the protoplasm of cells lining or filling the capsules of lacunar tissue. The whole of the lacunar tissue immediately underlying the prosomatic carapace is pigmented in this way (see this Journal, vol. xxiii, plate xii, fig. 34). The pigmentiferous property is by no means confined in either *Limulus* or *Scorpio* to connective tissue of the lacunar type. Networks of penetrating branched cells, containing pigment granules, occur in some parts, the "capsule" and "lacuna" having disappeared.

#### 4. RETICULAR CONNECTIVE TISSUE OF LIMULUS.

It is easy to understand how—by a reduction in the skeletal substance forming the incomplete capsules of lacunar tissue, so that it became a series of fine branched and reticulate cords, with here and there a cell resting on them—we could pass from

the lacunar tissue to a form of reticular connective tissue. Such reticular connective tissue consisting of branched or stellate cells, the branches being largely skeletal substance, with protoplasmic films overlying them in some instances, but not always—is found in *Limulus*, though rarely.

It occurs as “intrusive connective tissue” amongst the nerve-end cells of the central eyes (see this Journal, vol. xxiii, Pl. xii, fig. 27 *ss*), and here and there I have followed the lacunar tissue thinning out to such a reticular condition in other parts, e. g. in the neighbourhood of the great nerve-ganglia.

I have not found any connective tissue answering to this description in *Scorpio*, except so far as the branched pigmentiferous cells of the central eye agree with it.

##### 5. MEMBRANOUS CONNECTIVE TISSUE OF *LIMULUS* AND *SCORPIO*.

In both *Limulus* and *Scorpio* delicate ligamentous membranes occur here and there, which are formed by a special variety of connective tissue. This tissue is similar to the fibroid skeletal tissue, but differs in this, namely, that the skeletal substance is in the form of a thin, delicately fibrillated lamella, upon which are closely set the protoplasmic cells. These are often much flattened, and spread out on the surface of the membrane so as to constitute an endothelium (Pl. IX, fig. 3). But occasionally certain cells are found at intervals presenting a different character; these are plump, and have abundant granular protoplasm, similar to the cells drawn in Pl. XI, fig. 9. These exceptional cells proliferate, and are thrown off into the surrounding blood-fluid, probably as normal blood-corpuscles. Such membranous connective tissue forms the limit of the pericardial space in both *Limulus* and *Scorpio*. It also forms numerous ligaments, which pass from the pericardial wall to the heart. These ligaments have been described by Gegenbaur in the heart of *Limulus*<sup>1</sup>, who points out the mistake of Van der Hoeven, who had supposed them to be muscular.

<sup>1</sup> Gegenbaur. “Anatom. Untersuchung eines *Limulus*.” ‘Abhandlungen der Naturforsch. Gesellsch. in Halle.’ 1858.

Gegenbaur notices that these ligaments are elastic, and resist the action of reagents.

#### 6. LARGE-CELLED (EMBRYONIC) CONNECTIVE TISSUE OF SCORPIO.

In some regions, especially around the large pro-somatic ganglion in Scorpion, groups of cells, of oval or nearly spherical form, with abundant finely granular protoplasm, and often with two nuclei, indicating an active condition of multiplication, are found. They correspond in character to the little groups of proliferating cells above-mentioned as occurring in connection with the membranous connective tissue, but are numerous, and form a distinct tissue.

In these groups of cells (Pl. XI, fig. 9) we have the connective tissue reduced to a simple embryonic form. There is no skeletal substance developed around the protoplasm of the cells, but simply the protoplasmic units free from any special product of chemical metamorphosis. Possibly this tissue is concerned in the production of blood-corpuscles. Its cells are evidently in a high state of proliferous activity and closely resemble blood-corpuscles. It is possible to trace a gradation from the surrounding lacunar tissue to this large-celled tissue devoid of matrix or other ectoplasmic product and equally devoid of any entoplasmic product. The gradation is exhibited in the reduction of the capsular element of the lacunar tissue and the enlargement and close-setting of the protoplasmic cells (Pl. XI, fig. 10), until at last we find spots in which no capsular (skeletal or matrix product) is present at all and the protoplasmic cells are massed together in simple contact. The term 'embryonic' is appropriate to this tissue since at an early period when the special skeletal products of the connective tissue have not come into existence, it consists of indifferent embryonic cells packed side by side as in these small tracts surrounding the great nerve-ganglion. Here and there in other parts small quantities of this tissue are found. I have not observed this tissue in *Limulus*.



# 7. THE CARTILAGE-LIKE CAPSULIGENOUS CONNECTIVE TISSUE OF LIMULUS.

This is a very remarkable and interesting variety of connective tissue which occurs in one special organ of *Limulus* only, and is not found elsewhere in that animal nor in *Scorpio*. It is described by Gegenbaur as "cartilage" simply, but although agreeing with Vertebrate typical cartilage more closely than does the fibro-massive tissue of the entosternite, it yet presents some important points of difference from that tissue.

It occurs in a remarkable pair of ligamentous bands, the entapophysial ligaments which pass from one to another of the dorsal ingrowths of the integument known as the dorsal entapophyses. There is a right and a left series of these hollow chitinous peg-like ingrowths, and a ligamentous band to either series. The ligamentous band is not simply of equal dimensions throughout, but where it is attached to an entapophysis gives off at right angles a conical knob-like protuberance. A large series of muscles are attached to these protuberances.

These ligamentous bands, and their knobs, present in or when cut across a differentiation of substance: we distinguish at once an axial and a cortical tissue. The cortical tissue is identical with the fibroid tissue of the entosternite. The axial tissue is the peculiar capsuligenous tissue now to be described. In Pl. XI, fig. 4, the junction of the two tissues is seen. Nowhere do we find the capsuligenous tissue isolated; it is always surrounded by a thick coating of the fibroid entosternal tissue. Occasionally small groups of cells belonging to the capsuligenous tissue become detached from the axial mass, and may then be seen isolated in a matrix of fibroid tissue. Single cells and groups of cells in various stages of development may be observed in this condition, which enables the observer to detect the law of their growth more readily than when the cells are closely pressed together in the large mass of axial tissue.

The characteristic feature of the capsuligenous tissue consists in the fact that each cell surrounds itself (as in typical carti-

lage), as soon as formed by division of a previous cell, with a definite, refringent, and firm capsule. The capsule of the mother-cell thus becomes fitted internally with two secondary capsules, each containing a nucleated corpuscle of protoplasm. The process is repeated, and each time the products of division form for themselves new, complete capsules, which, on account of their differing refractive properties, can be readily distinguished. The outermost capsules enlarge as the protoplasm within increases in size, and gives rise to capsule within capsule. The general disposition of these capsules is precisely like that of the capsules formed by actively vegetating specimens of the Alga *Glaucocapsa*, or by some specimens of Vertebrate cartilage. The

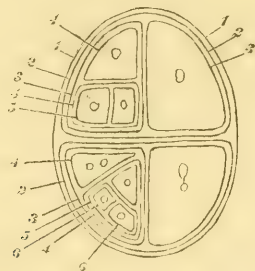


FIG 1.

accompanying woodcut (fig. 1) shows the arrangement of successive generations of capsules, the numerals indicating the age of each capsule. This figure is copied from Gegenbaur's memoir. It gives, in a diagrammatic form, the condition attained by an isolated mother-capsule. After a certain size has been attained, the outermost capsule breaks down, and the single group arranges itself as two or four groups. But where the capsules are closely pressed against one another in the main tract of the axial tissue of the entapophysial ligament, there is an irregularity of form produced, and an irregularity of growth which renders it difficult to trace clearly the boundaries of cell-groups.

Moreover, there is not present in the axial tract of this tissue

anything corresponding to the general matrix of a hyaline cartilage. The capsules are all closely packed, without giving rise to any quantity of homogeneous inter-capsular substance.

They resemble in this the cell-walls of vegetable parenchyma. In another respect, also, the capsules resemble vegetable cells, and differ from typical Vertebrate cartilage. The protoplasm does not fill its capsule. A large cavity unoccupied by protoplasm exists in all the larger capsules (Pl. XI, fig. 4, *c*). Further, just as vegetable cell-walls break down, so as to place neighbouring cells in communication, and thus constitute ducts and vessels, so here do we find the walls of the capsules often incomplete, probably always becoming so after a time (Pl. XI, fig. 7). Thus the space or cavity unoccupied by protoplasm in each capsule is placed in continuity with the similar space in neighbouring capsules. These communications are not, as the comparison to vegetable ducts might lead it to be inferred, arranged in a straight line, but, in accordance with the triaxial growth and expansion of the tissue, they are developed in all directions.

The protoplasm lying within each capsule is disposed all round the capsule as a "primordial utricle," and fills usually about half its cavity. The space not occupied by the protoplasm is occupied by a thin liquid common to the whole system of communicating capsules. There is no special feature calling for remark about the nuclei, the character of which is seen in the drawing, Pl. XI, fig. 4.

Where the capsuligenous tissue is in contact with the fibroid tissue of the cortical layer of the entapophysial ligament it is seen that the substance of the capsule is continuous with the intercellular fibroid matrix of the fibroid tissue (Pl. XI, fig. 4 *R*).

A little reflection renders it evident that the plan of structure of the capsuligenous tissue is essentially the same as that of the lacunar connective tissue above described (Sect. 3). Were the capsules of the capsuligenous tissue much larger, and the skeletal substance of which they are composed less dense and more delicate, and were the proliferating cell-units of pro-

toplasm less active in enclosing themselves (as soon as they are formed by fission) in a new complete investment of capsule substance, so that several cell units were disposed around the concave wall of a capsule, we should have the condition which characterises the lacunar tissue.

The difference lies merely in this, that capsule-formation proceeds less energetically in the lacunar than in the capsuligenous tissue; in the former the skeletal capsule-forming product is only produced in such a way as to enclose several cells; in the latter every cell completes or nearly completes a capsule for itself. The space or lacuna in the centre of each group of cells enclosed by the trabeculae of the lacunar tissue (see Pl. X, fig. 1 *e*) is the same thing as the space unoccupied by protoplasm within the capsule of the capsuligenous tissue, and in both cases the space of one capsular area is in communication with neighbouring similar spaces, so as to form a spongy or reticular lacunar system.

Whether this system is (as seems probable) in communication with the blood-vessels and large blood-lacunae I am unable to decide in reference to the capsuligenous tissue, equally as in reference to the lacunar tissue.

Like the lacunar tissue, the capsuligenous appears to be penetrated by small blood-vessels, which lie between the capsules (Pl. XI, fig. 4 *x*). That these structures (*x*) are blood-vessels is, however, doubtful.

It is stated by Gegenbaur that this capsuligenous tissue is found also in the hollow gill-laminae of *Limulus*, forming the substance of the numerous "pillars" which unite the two membranes of which each lamella consists. I have not succeeded in finding this tissue in that position.

I have not made a special chemical examination of the capsuligenous tissue.

#### 8. THE BLOOD-CORPUSCLES OF *LIMULUS* AND *SCORPIO*.

There is a very striking agreement between *Limulus* and *Scorpio* in the form, size, and granulation of their blood-corpuscles, as also in the appearance of the blood when shed.



The blood of *Limulus* contains a large quantity of Fredericq's hæmocyantin, so that it may exhibit a deep indigo tint when in quantity. The same is true of the Scorpion's blood. When obtained in bulk—as I have obtained it from large specimens of *Androctonus funestus*—the blood exhibits as deep a shade of indigo blue as does that of *Limulus*.

The blood-corpuscles of *Limulus* and of two species of Scorpion are drawn in Pl. VIII, figs. 4, 5, 6. In measurement they closely agree, being of unusually large size for Arthropods. Usually, when shed, they exhibit an oval shape, and the more elongated examples are as much as  $\frac{1}{1000}$ th inch long. In both *Limulus* and *Scorpio* the corpuscles are sometimes seen with irregular amœboid processes, and when observed in the living state in the gill laminae of *Limulus* they very generally have an irregular outline, and may be seen to undergo changes of form.

In both *Limulus* and *Scorpio* the blood-corpuscles are remarkable for containing a number of coarse granules, larger and more abundant in the latter than the former.

The exact nature of these granules I have not determined. They are highly refringent, and appear to be similar in substance with the granules observed in the protoplasm of the cells of the lacunar tissue (see above, Section 3).

## 9. THE COXAL GLANDS OF THE ARACHNIDA.

In No. 221 of the 'Proc. Roy. Soc.' (June 15th, 1882), I described and figured the position of certain glandular bodies—the "coxal glands," as I termed them—occurring in the prosoma of *Scorpio* and its subgenera. The woodcut showing the position of these glands is here reproduced.

I showed that these bodies had been erroneously supposed by Newport and others to be part of the alimentary canal; at the same time I identified with them the "brick-red glands" discovered by Packard in *Limulus*. I now submit a woodcut copy of Packard's figure of the brick-red gland of the right side of *Limulus polyphemus* for comparison. It will be observed that the position of the gland is closely analogous to

that occupied by the coxal gland of Scorpions—namely, in that region where the coxæ of the prosomatic limbs join the sternal

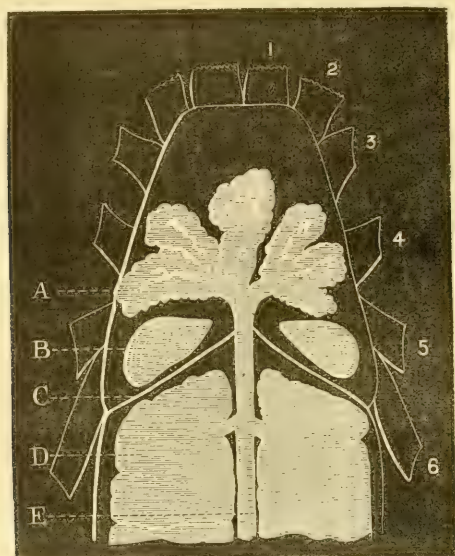


FIG. 2.—Diagram of the anterior portion of a Scorpion's body to show the position of the coxal glands. A. Anterior glandular cæca of the alimentary canal (salivary glands of Newport and Blanchard, not of Dufour). These are drawn of smaller size than natural, and are turned forward so as to expose the coxal glands. B. The coxal gland of the left side. C. Fibrous septum (diaphragm of Newport) formed by the posterior flaps of the entosternite. D. Glandular cæca of the alimentary canal (so-called "liver"). E. Axial portion of the alimentary canal. 1 to 6. The six pairs of limbs of the prosoma.

wall of the prosoma. Instead, however, of presenting us with a single oval gland in relation to the coxa of the sixth prosomatic limb, *Limulus* shows four lobes appropriate to the second, third, fourth, and fifth limbs of the prosoma respectively.

I mentioned in the paper quoted that I had found a similarly situated coxal gland on each side of the entosternite in a large South American *Mygale*, and that in this genus the gland presented lobes corresponding to the coxæ of the prosomatic

limbs as in *Limulus*, instead of being a simple oval body as in *Scorpion*.

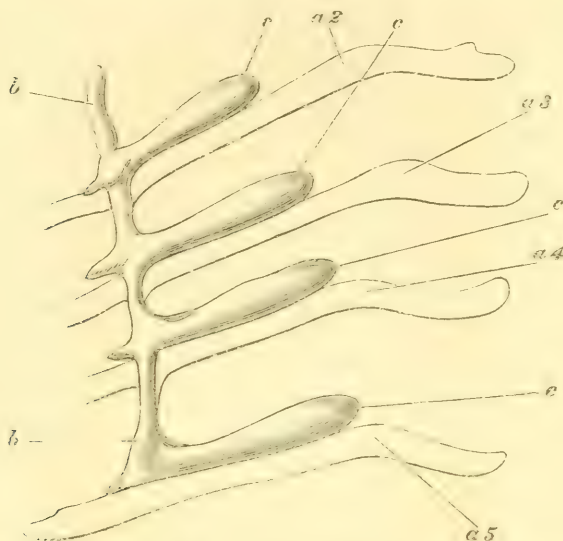


FIG. 3.—Right coxal gland of *Limulus polyphemus*, Latr., after Packard ('Anniversary Memoirs of the Boston Society of Natural History,' 1880), *a2*, *a3*, *a4*, *a5*. Posterior portion of the chitinous base of the coxæ of the second, third, fourth and fifth prosomatic limbs. *b*. Vertical stolon of the gland. *c*. Its four transverse lobes, coinciding respectively with the second, third, fourth and fifth coxæ.

I have not had an opportunity of dissecting the coxal glands fully in *Mygale* at present, but I have found them to be present in *Mygale cementaria* (the trap-door spider), kindly sent to me from Naples by Mr. Neville Reid. In the common English *Epeira diadema* I have failed to find evidence of the presence of the coxal glands in complete series of microscopic sections cut transversely to the long axis of the prosoma, and I am inclined to conclude that they are absent in the smaller spiders.

The discovery of these glands in other *Arachnida* such as *Thelyphonus*, *Galcodes*, and *Opilio* offers an interesting field for future investigation.

In the paper referred to I have given a brief account of the minute structure of these coxal glands in *Scorpio* and *Limulus*, and have pointed out the inconclusive nature of the statements made with regard to the structure of the coxal glands of *Limulus* by Dr. Packard. In *Limulus* Dr. Packard was unable to find any duct by which the coxal glands open to the exterior, and I have equally failed to find any such duct in *Limulus*, *Scorpio*, or *Mygale*. Although a negative conclusion of the kind must be accepted with some reserve, I am inclined to believe, as the result of a more extended study of these glands by means of complete series of transverse sections of the prosoma in *Scorpio*, that the coxal glands are devoid of any duct opening either to the exterior of the body or in any other way. They appear to be ductless glands, but possibly further study of them may show that in early life or at some unexpected point they do possess a duct.

In minute structure the coxal glands of *Scorpio*, *Mygale*, and *Limulus* present a definite agreement with one another, whilst showing each remarkable peculiarities of structure.

The nature of these agreements and differences will be best set forth by a description of the structure of each in sequence, and by the comparison of the illustrative figures in Plates XI and XII, which accompany this memoir.

#### 10. STRUCTURE OF THE COXAL GLANDS OF SCORPIO.

In transverse sections of the prosoma of *Scorpio* about the region of the meta-sternum (the pentagonal or triangular cuticular sternal plate of *Buthus* and *Androctonus* respectively) the coxal glands are seen occupying the position indicated in the accompanying diagram fig. 1 (woodcut). This diagram also serves to show the position of the body  $\tau$ , and sub-neural portion  $u$ , of the entosternite and its relation to the coxal glands.

In such a section it is seen that the cavity of the prosoma is very closely packed with muscles, caeca of the gastric gland (those marked A in the woodcut fig. 2) and the coxal glands s.

In several complete series of such sections made through the prosoma of specimens of *Scorpio Italicus* I have failed to find



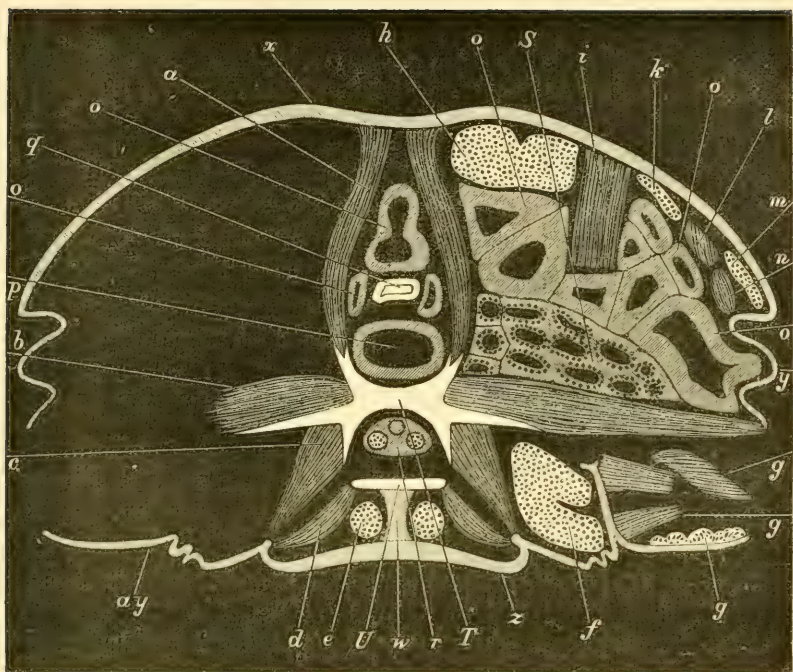


FIG. 4.—Diagram of a transverse section of the hinder part of the prosoma of the Italian scorpion (*Euscorpium italicus*, Roess.) passing through the pentagonal metasternal plate *z*, and showing the relations of the entosternite *T*, its sub-neural arch *U*, the coxal glands (of the right side) *S*, and the gastric cæca *o*. It is important to note that the spaces left between various organs are in some cases natural blood-sinuses, in other cases they are due to shrinking in the process of preparing the section. It is difficult to determine which are natural and which shrinkage spaces, but for the present purpose this is immaterial. The following is a complete list of reference letters.—*a*. Tergo-plastral muscle (from tergal tegumentary sclerite to the entosternite or "plastron"). *b*. Pleuro-plastral muscle. *c*. Sterno-plastral muscle. *d*. Sterno-hyponeural muscle. *e*. Longitudinal pectoral muscle in transverse section. *f*. Muscle in the coxa of the sixth prosomatic limb in transverse section. *g, g, g*. Muscles in the coxa of the fifth prosomatic limb. *h*. Dorsal longitudinal muscle in transverse section. *i*. Part of a dorso-ventral muscle, cut parallel with its fibres. *k*. Similar to *h*. *l*. Similar to *i*. *m*. Similar to *h*. *n*. Similar to *i*. *o*. Cæca of the gastric gland in section. *p*. The axial portion of the alimentary canal. *q*. The anterior aorta. *r*. A mass of connective tissue investing the two nerve-cords (right and left) and the supra-medullary artery (median). *S*. The right coxal gland. *T*. The body of the entosternite. *U*. The subneural arch of the entosternite (the section is taken at a plane in front of the connection of these two parts of the entosternite). *w*. Tissue connecting the subneural arch of the entosternite with the tegumentary metasternal (pentagonal) plate *z*. *x*. Chitinous sclerite of the tergum. *y*. Soft tegumentary membrane forming the sides of the body and connecting the sclerites of the dorsal and ventral surfaces. *z*. The pentagonal metasternite in section: immediately adjacent to it is the coxa of the sixth prosomatic limb containing a muscle marked *f*, beyond this again is the coxa of the fifth prosomatic limb, with muscles marked *g*. The sclerite of the coxa of the fifth prosomatic limb on the left side is marked *ay*.

evidence of any duct to the coxal gland, either communicating with the exterior or with the alimentary canal or with any blood-space.

The structure of the coxal gland of *Scorpio* is shewn in Pl. VIII, fig. 7, and in Pl. XII, fig. 5. In the first of these figures we have a complete section through the gland as seen under a low power of the microscope.

We distinguish

1. The medullary substance.  $\kappa$  in Pl. VIII, fig. 7.
2. The cæca of the gland,  $\kappa$ .
3. The inter-cæcal spaces lined by extensions of the medullary tissue,  $o$ .

The medullary tissue is a compact variety of the "lacunar tissue" above described (sect. 3). In the central core of medullary substance it is solid with few if any lacunæ between the constituent cells, but in the intercæcal passages it opens out so as to leave a wide space bounded and occasionally traversed by a sparsely developed connective tissue (see Pl. XII, fig. 5,  $o$ ,  $m$ ).

The central core of medullary tissue ( $\kappa$  Pl. VIII, fig. 7) is traversed by a blood vessel *cbv*, and presumably the contents of this blood vessel are in communication with the lacunar space of the inter-cæcal regions of the gland.

The intercæcal spaces and connective tissue are liable to be misapprehended on first examination. The connective tissue is so inconsiderable in amount, its nuclei so small in size, and the "lacunar space" so large, that one is apt to overlook altogether the connective tissue frame-work and to see only the spaces or channels surrounding the glandular cæca. Staining the sections with Borax Carmine, however, suffices to render the connective tissue framework obvious enough, as in Pl. XII, fig. 5,  $m$ . I have not observed any coagulum nor any free corpuscles in the intercæcal lacunæ.

The cæca of the gland appear in sections as circular or oval spaces lined by a single layer of large cells which constitute the special and characteristic epithelium of the coxal

glands. I have not been able to trace the connections of these spaces and cannot assert definitely that they are not tubular, but I am inclined to regard them as brought about by the subdivision of an originally simple sac, the walls of which have given rise to in-growing trabeculae which have more or less completely united so as to divide the original simple lumen of the gland into a number of compartments.

The cells themselves (Pl. XII, fig. 5, *ln*, *lp*.) which line these compartments are remarkable in the first place for their size and for that of their nuclei. Their dimensions are shewn in Pl. XII. Secondly, they are remarkable for the great development of a cortical layer *cr*, which is finely striated or rather tubulated.

This cortical layer is developed on the surface of the cells turned away from the lumen of the gland, that is, on the surface which is in relation to the lacuniferous connective tissue. In sections which cut this surface of the cells (that is, tangential to the caeca) as in fig. 2, Pl. XII, they may appear each surrounded by a ring of cortical striated substance owing to the fact that the cortical matter dips in some parts between neighbouring cells, though it never descends so far around the cell as to reach the inner surface of the protoplasm (that which bounds the lumen of the glandular caeca). The protoplasm of the cells beneath the highly refringent cortical layer, is very delicate, transparent and with a few scattered coagula when prepared in section. A coagulum is not unusually found within the lumen of the glandular caeca (Pl. XII, fig. 5, *kc*) and whether normally or as the result of preparation, one finds occasionally the large nuclei of the gland epithelium detached from the cell-protoplasm and involved in this coagulum.

## 11. STRUCTURE OF THE COXAL GLANDS OF MYGALE.

The coxal glands of *Mygale* present the same general structure as those of *Scorpio* excepting that in them the central core of medullary connective tissue does not occur. Instead of a series of inter-caecal spaces with sparse lacunar connective tissue, we find in *Mygale* the inter-caecal spaces completely



filled by the remarkable colloid homogeneous substance which I have mentioned above as occurring in certain fissures in the entosternite of that animal. Small nuclei which stain pink with carmine, and are surrounded by a delicate envelope of unstained protoplasm are scattered in isolated positions throughout this colloid matrix. The tissue so constituted I have termed above, colloid tissue. (Pl. XII, fig. 1, *col.*) There is thus then in *Mygale* no space which could serve as a blood-carrying space between the cæca of the gland.

The cæca of the gland are larger than in *Scorpio*, they have an irregular outline and are often greatly compressed, that is, have a long, narrow and irregular lumen. The cells which line these cæca are of great size, very much larger than the corresponding cells in *Scorpio*. The measurements are given on Plate XII. Just as in *Scorpio* so here we find a strongly refringent, dense, cortical substance developed on the deep surface of the gland cells (Pl. XII, fig. 1, *cr.*) where they rest on the colloid tissue. The protoplasm is soft and delicate and easily tears away from this dense cortical layer. The gigantic nuclei are frequently to be seen in sections lying loose in a coagulum in the lumen of the glandular cæca.

## 12. STRUCTURE OF THE COXAL GLANDS OF *LIMULUS*.

At first sight the coxal glands of *Limulus* do not seem to be constructed on the same type as those of *Scorpio* and *Mygale*. A great difference in appearance is brought about by the fact that in *Limulus* the intercæcal connective tissue is very abundant, and forms the bulk of the gland (Pl. XII, fig. 4; *a, b, c*), whilst the cells which constitute the layer lining the glandular cæca are not large cells, but have nuclei of the same size as those of the intercæcal connective tissue, and no excess of protoplasm. Accordingly the observer does not readily draw a distinction between the gland epithelium and the surrounding connective tissue. Indeed, to distinguish between them is often very difficult, for the intercæcal connective tissue presents numerous lacunæ (which resemble those of the usual lacunar tissue), and are set round with cells in the



same way as are the gland-cæca, so that it is not always possible in a section to say which is a glandular cæcum, and which is a connective-tissue lacuna. (See the right hand side of fig. 1 in Plate X, in illustration of this fact.)

The fact also that there is no definite membranous capsule to the coxal glands of *Limulus*, but that the lacunar tissue of the prosomatic mass passes directly into the intercæcal tissue of the gland, as shown in Plate 10, fig. 1, at the line *nn*, renders it not improbable that the whole gland—intercæcal tissue and cæcal glandular epithelium also—is a modification of the connective tissue. It will be seen by Plate X, fig. 1, how difficult it is to distinguish the lacunæ *e* and the glandular cæca *k*. Possibly this is a true suggestion as to the origin of the glandular epithelium, both in the coxal glands of *Limulus* and in those of *Scorpio* and *Mygale*; possibly it is in all three a gland-cell modification of the connective tissue. But until it is proved by embryological research that this is the case, I shall adopt the more probable hypothesis that the glandular epithelium is an offspring of either the hypoblast or the epiblast, and that its cæcal growths are packed in and supported by mesoblastic tissue—the intercæcal element in all three cases alike.

The cæcal epithelium in *Limulus*' coxal gland, when favorably seen, presents distinctive characters. Its nuclei are round (those of the intercæcal tissue are often oval) and it has the same deeply placed, highly refringent, finely striated, cortical layer which characterises the cæcal epithelium of the coxal glands of *Scorpio* and *Mygale*. The cells being much smaller in *Limulus* than in the latter, the cortical layer is proportionately narrow, but it is sufficiently obvious to give a strong limiting line to the cell-layer on the side towards the connective-tissue framework (Pl. XII, fig. 4; *cr*). It is also striated as in *Scorpio* and *Mygale*.

The form of what I have called the "cæca" in *Limulus* for the sake of comparison with *Scorpio* and *Mygale* differs very much from the spaces so-called in these latter forms. The substance of the coxal gland of *Limulus* is, in fact, honey-combed by an irregular series of chambers—apparently all

forming one system, and it is these irregular spaces which are lined by the glandular corticated epithelium. In a section seen with a low power, these passages (cæca) present the appearance shown by the white spaces in Plate XI, fig. 5, but it must be remembered that in a thickish section the passages are seen to bend and twist, anastomosing with one another, and leaving broad solid trabeculæ between them.

The centre of each lobe of the coxal gland of *Limulus* (woodcut, fig. 3, *c*) is occupied by a blood-vessel, around which the lacuniferous connective tissue is disposed in concentric layers, into which tissue a few out-growths of the cæcal system (the subdivided gland-lumen) push their way. As we recede from the axis of the lobe the glandular spaces become more abundant and the substance therefore spongy, until again at the periphery the glandular spaces cease, and the compact form of connective tissue (Pl. X, fig. 1 ; 1) clothes the surface of the lobe and passes over rapidly into the form of normal lacunar tissue (see Pl. X, fig. 1 ; 11).

The intercæcal connective tissue is far more abundant in *Limulus* than it is in *Scorpion*: it resembles that of the latter far more closely than it does the colloid tissue of *Mygale*. It is clearly enough only a local modification of the "lacunar tissue" which packs the cæca of the gastric gland. Its lacunar spaces are often large, but the protoplasmic corpuscles which often closely line those of normal lacunar tissue, are here more sparsely developed. The skeletal element also presents a distinct character. It tends to develop long fibre-like cords of a highly refringent character which must give a certain strength and elasticity to the framework of the gland (see Pl. XII, fig. 4 ; *b*). There is no direct transition from this intercæcal tissue to the lacunar tissue, the periphery of each lobe being, as above pointed out, formed by a special variety of connective tissue in which the skeletal element is almost entirely absent, and the protoplasmic bodies are set closely in a compact mass (Pl. X, fig. 1 ; 1).

Packard has already drawn attention to the red colour of the coxal glands of *Limulus* in the fresh state. This colour led

him to give to them the name of "the brick-red glands." This colour they lose when placed in alcohol. If a section of the gland is examined in the fresh state, it is found that the red colour is due to the presence of numerous small red-coloured granules which occur in the layer of gland-epithelium. A piece of this epithelium, as seen in the fresh state without the addition of any reagent, under a No. 10 immersion of Hartnack, is shown in Plate XI, fig. 6. The red granules are there presented with their natural colour. In this fresh condition the nuclei of the gland-cells are not seen, owing to the refractive properties of the living tissue.

## 12. GENERAL CONCLUSIONS AS TO THE COXAL GLANDS OF SCORPIO, MYGALE, AND LIMULUS.

The minute structure of the glands above recorded leaves little room for doubt that we have in the coxal glands an active secretory apparatus. The materials upon which the gland-epithelium acts are probably brought to it by the intermediary of the intercæcal tissue, and the product of secretion is accumulated in the lumen of the gland-cæca. But it must be admitted that no abundant or peculiar-looking secretion can be detected in this lumen—only in some cases a colourless coagulable material—and further, it seems that there is no outlet for this secretion.

On the whole, the facts seem to favour a comparison with the green-glands (antennary coxal glands) of the Decapod Crustacea. But these glands have a definite outlet, and also show a different structure in their gland epithelial cells, as seen in the portion of the green gland of *Palæmon*, figured in Pl. VIII, fig. 8. These latter have no trace of the fibrillated cortical substance which characterises the gland-cells of the coxal glands equally in *Scorpio*, *Mygale*, and *Limulus*.

It seems to me that, until we have embryological evidence to the contrary, we must still hold the possibility before our minds of the development of these coxal glands of *Arachnida* from skeleto-trophic tissue. They may be, from beginning to end, a product of the differentiation of such tissue, like some

of the ductless glands of Vertebrata. On the other hand, it is possible (and more probable) that whilst their intercæcal framework is developed from skeleto-trophic tissue, the lining layer of cells of their glandular cæca is an offset either of the epiblast or of the hypoblast (that is, either of the epidermal cell-layer or of the enteric cell-layer).

In any case, the occurrence of these glands in their characteristic position, and with their characteristic corticated secretory cells in *Limulus* on the one hand, and in *Scorpio* and *Mygale* on the other, is one more argument in favour of the classificatory association of *Limulus* with the Arachnida.

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## **An Improvement in the Method of using the Freezing Microtome.**

By

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THE process of obtaining thin slices of soft structures by means of embedding in paraffin has now been brought to a state of almost ideal perfection ; on the other hand the method of "freezing" still remains almost in its infancy. At present it is only with great trouble that a continuous series of slices can be obtained with it, and if these are cut from a loose disconnected tissue, they break up immediately on being introduced into water to free them from the gum in which they are always embedded. Moreover, the waste of time involved in transferring from water to a glass slide is simply appalling.

Yet the freezing process has special advantages of its own.

In the case of many tissues it affords a clearer insight into structure ; perfect staining is not so indispensable (provided, as is usually the case, glycerine be used as a medium for mounting) ; and when hard parts occur in a preparation along with soft, both may be evenly cut through with equal ease. It is not likely, therefore, to fall wholly out of use, particularly for certain refined histological work, and improvements may be confidently expected.

The following may perhaps be regarded as a first step to others. Instead of freezing in gum, as is usual, one uses gelatine jelly. This is prepared and clarified in the ordinary manner. It should set into a stiff mass when cold ; how stiff will best be learned by experience.

The tissue to be cut is transferred from water to the melted jelly, and should remain in it till well permeated.

It is then placed on the piston of a Rutherford's microtome; the "well" should not be filled: for adherence it is sufficient to roughen the surface of the piston with a file. No more jelly should be used than is sufficient to surround the specimen; if too much has been added, it may be removed when frozen by careful paring.

When well frozen, slices may be cut in the ordinary way; while frozen they should be quickly transferred to the glass slide on which they are to be mounted. On touching the glass, the slice of jelly almost immediately thaws and adheres as a consistent fibre to the surface. When enough slices have been placed on the slide, they should each be covered with a drop of glycerine (the sooner this is added the better); a cover glass is then superposed, zinc white or some similar cement is run round it, and the preparation is complete. In process of time the glycerine will permeate the gelatine and convert it into glycerine jelly; if this does not take place soon enough, it may be hastened by placing in an oven kept at a temperature of about  $20^{\circ}$  to  $30^{\circ}$  C.

In this way a series of entire slices of great thinness may be obtained from the most disconnected structures; even when they contain hard silicious spicules, as in the case of sponges.

Diatoms may be cut without difficulty by this method; I have now beside me some slices of *Pleurosigma*, which reveal the internal anatomy of these organisms in an admirable fashion. It need not be added that the process effects a considerable saving in labour and time.

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## EXPLANATION OF PLATE I,

Illustrating Messrs. Carpenter and Sladen's papers on the  
"Apical System and Primary Larval Plates of Echinoderms."

The following lettering is used throughout all the figures :

- i. Dorso-central. 2. Under-basals. 3. Basals (shaded dark). 4. Radials.  
5. Orals. 1. Primary interbrachial plate. r. Radial shield. t. Terminal.  
an. Anus. w. p. Water-pore, or madreporite.

FIGS. 1—9.—A series of figures representing portions of the disc of different Ophiurids, to illustrate the variations in the development of the apical system, i. e. the rosette of primary plates. All after Lyman.

1. *Ophioceramis clausa*. 2. *Ophiomusium validum*. 3. *O. flabellum*. 4. *Ophioglypha lapidaria*. 5. *Ophiomitra exigua*.  
6. *Ophiozona antillarum*. 7. *Ophiomusium granosum*.  
8. *Ophioglypha minuta*. 9. *Ophiomusium pulchellum*.

FIGS. 10—16.—A series of diagrams representing the primitive plates in the larvæ of Brachiote Echinoderms, an early and a later stage in the larva in each of the orders being given.

FIG. 10.—Abactinal aspect of a young Crinoid (*Antedon rosacea*) shortly before detachment from the stem takes place. After Dr. Carpenter.

FIG. 11.—Abactinal aspect of an early Crinoid larva (*Antedon rosacea*), in which the radials are still very small. The stem-joints are represented as telescoped into one another, so as to bring the terminal plate of the base of the stem or dorsocentral (1) into close relation with the basals (3). From P. H. Carpenter.

FIG. 12.—Abactinal aspect of a young Ophiurid (*Amphiura squamata*). After Ludwig, slightly altered.

FIG. 13.—Abactinal aspect of an early Ophiurid larva (*Amphiura squamata*). From P. H. Carpenter, after Ludwig.

FIG. 14.—Abactinal aspect of a young Asterid (*Asterina gibbosa*).

FIG. 15.—Abactinal aspect of an early Asterid larva (*Asterina gibbosa*). From P. H. Carpenter, after Ludwig.

FIG. 16.—Abactinal aspect of a young Asterid (*Zoroaster fulgens*).

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## EXPLANATION OF PLATES II & III,

Illustrating Mr. Sedgwick's Paper on the "Origin of Metameric Segmentation."

### *Complete List of Reference Letters.*

*A.* Anus. *a.* Anterior end of young embryo. *A. P.* Abdominal pore. *B.* Body-wall. *C. H.* Cerebral hemisphere. *C.* Longitudinal canal connecting pouches of archenteron. *E.* Ectoderm. *G.* Gill pouch. *H.* Heart. *K.* Nephridium. *K. D.* Longitudinal duct of Nephridia (segmental duct). *M.* Mouth. *M. A.* Coalesced part of primitive mouth. *ME.* Mesenteron. *me.* Edge of mesenteries. *M. S.* Mesoblastic Somites. *N.* Nervous system between mouth and anus. *N<sup>1</sup>.* Præoral part of nervous system. *N<sup>2</sup>.* Postanal part of nervous system. *N. C.* Neural canal. *Ne.* Posterior opening of neural canal. *O.* External openings of Nephridia. *P.* Pouches of archenteron. *P.* Præoral lobe. *Py.* Anterior opening of neural canal. *Si.* Siphonoglyphe. *Si'.* Upper end of siphonoglyphe projecting beyond general edges of lips. *St.* Wall of stomodæum.

Figs. 1—5.—Five young embryos of *Peripatus capensis*, ventral view. From drawings by Miss Balfour. *a.* Denotes the anterior extremity.

FIG. 1.—Youngest embryo, with slightly elongated blastopore.

FIG. 2.—Embryo, with three somites and elongated blastopore.

FIG. 3.—Embryo, with five somites. The blastopore is closing in its middle portion.

FIG. 4.—The blastopore has completely closed in its middle portion and given rise to two openings, the future mouth and anus. The primitive streak is deeply grooved.

FIG. 5.—Embryo, with about thirteen somites; flexure of hind part of body commenced. The remains of the original blastopore are present, as the mouth placed between the second pair of somites, and the anus placed on the concavity of the commencing flexure of the hind part of the body.

FIG. 6.—Stomodæum of *Peachia*, laid open so as to show the siphonoglyphe. This figure was very kindly drawn for me by Mr. W. F. R. Weldon. *T.* Tentacles. *St.* Wall of stomodæum. *Si.* Siphonoglyphe. *Si'.* Upper end of siphonoglyphe, projecting beyond the general edges of the lips. *B.* Body-wall. *me.* Edge of mesenteries.



## EXPLANATION OF PLATES II & III—continued.

FIG. 7.—Diagram of ideal ancestor of segmented animals, viewed as a transparent object from the ventral surface. *A.* Central part of archenteron. *P.* Pouches of archenteron (four represented on either side). *C.* Longitudinal canal connecting pouches. *O.* Excretory pores. *N.* Nervous ring. *M. A.* Dumb-bell shaped mouth. Ectoderm.

FIG. 8.—Diagram showing Invertebrate arrangement. Archenteric pouches separate from central part of archenteron (now called mesenteron). *E.* Ectoderm. *M. E.* Mesenteron. *M. S.* Mesoblastic somites. *K.* Nephridia. *O.* External openings of Nephridia. *M.* Mouth. *A.* Anus. *M. A.* Coalesced medium part of primitive mouth. *N.* Central nervous system; dumb-bell shaped like that of *Peripatus*. Wall of mesenteron, yellow. Mesoblastic somites, blue. Nephridia, red.

FIG. 9.—Diagram of Vertebrate arrangement from neural (dorsal, i.e. ventral of Invertebrata). Excretory pores are not developed, except behind, *A. P.*; and in front, *G.* Colours and letters as in Fig. 8, except *G.*, gill pouch. *K. D.* Longitudinal duct of Nephridia, or segmental duct, opening behind into mesenteron. *A. P.* Pore retaining Invertebrate arrangement = abdominal pore. Mouth, anus, and nervous system not shown.

FIG. 10.—Diagrammatic transverse section through Invertebrate. Colours as before. *M. S.* Somite. *M. E.* Mesenteron. *N.* Nervous system. *K.* Nephridia.

FIG. 11.—Diagrammatic transverse section through Vertebrate. Colours as before. *N. C.* Neural canal. *M. E.* Mesenteron. *K. D.* Segmental duct. *K.* Segmental tube (nephridium). *M. S.* Somite.

FIG. 12.—Diagram of longitudinal vertical section of Invertebrate. Vascular system, red. *P.* Præoral lobe (hæmal). *H.* Heart. *N.* Nervous system. *N<sup>1</sup>.* Præoral nervous system. *N<sup>2</sup>.* Postanal ditto. *M. E.* Mesenteron. *M.* Mouth. *A.* Anus.

FIG. 13.—Diagram of ideal intermediate type, with terminal mouth and anus. Letters and colours as in Fig. 12.

FIG. 14.—Diagram of arrangement of *Balanoglossus*, with neural præoral lobe and without præoral and postanal nervous system.

FIG. 15.—Diagram of arrangement of embryo Ascidians and Vertebrata. Nervous system folded in. (Siphon stage.) *Py.* Anterior opening of neural canal (site of the pituitary body). *Ne.* Posterior ditto. *N. C.* Neural canal.

FIG. 16.—Diagram of Vertebrate arrangement. *C. H.* Cerebral lobe.

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### DESCRIPTION OF PLATE IV,

Illustrating Mr. A. G. Bourne's paper "On Certain Abnormalities in the Common Frog (*Rana temporaria*)."

FIG. 1.—Urogenital organs, ventral view. *herm.* Ovotestis. *te.* Testicular portion. *ov.* Ovarian portion. *k.* Kidney. *ur.* Ureter. *cl.* Cloaca. *C. Ad.* Corpus adiposum.

FIG. 2.—The ovotestis, dorsal view. *te.* Testicular portion. *ov.* Ovarian portion. *m.or.* Mesorchium. *m.o.* Mesovarium.

FIG. 3.—Ovotestis from *Rana temporaria*, section passing through the junction of testicular and ovarian portions. *ov.* Ova. *sp.* Seminal crypts.

FIG. 4.—Section passing through the junction of the testis, with the rudimentary ovary in *Bufo cinereus*. *ov.* Cells resembling ova. *sp.* Seminal crypts.

FIG. 5.—Dorsal view of normal vertebral column of *Rana* (after Ecker).

FIG. 6.—Dorsal view of abnormal column, Case A.

FIG. 7.—Ventral view of atlas vertebra, Case A.

FIG. 8.—Ventral view of 3rd vertebra, Case A.

FIG. 9.—Dorsal view of abnormal column, Case B.

FIG. 10.—Dorsal view of 10th vertebra, Case B.

FIG. 11.—Vertical elevation of same vertebra, from behind.

FIG. 12.—Urostyle of same frog, showing the concave facet as in the normal urostyle.

References in Figs. 5—12 :—*at.* Atlas vertebra. *ax.* Axis vertebra. *ur.* Urostyle. 1—10. Numbers of the vertebræ.

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### DESCRIPTION OF PLATE V,

Illustrating Mr. Poulton's Paper on "The Structures connected with the Ovarian Ovum of Marsupialia and Monotremata."

FIG. 1.— $\times 9$ . Transverse section of the ovary of *Phalangista*. The figure gives a representation of the appearances seen in an average section of this ovary. Even under this low power there are certain obvious differences between such a section and that of the Mammalian ovary as usually described. This is especially true of the larger follicles (*F*. and *F'*.), of which the membrana granulosa (*M. G.*) forms a very thin and regular layer, only marked by slight thickenings at the point nearest to the ovum (*o*). The latter is never embedded in the membrana granulosa in the largest follicles, so that the slight thickenings are the only indications of a cumulus proligerus, and the ovum lies in the abundant contents of the follicle. Other differences will appear in the more highly magnified figures. *st.* Stroma, consisting of fibrous tissue and stroma cells. *F*. Large follicle, with the included ovum (*o*) turned towards the outside of the ovary. *f. t.* Tunica fibrosa, becoming highly vascular close to the follicle. *m. g.* Membrana granulosa, relatively thin and regular (except near to the ovum). *i. l.* Intermediate layer, in which the cells of the membrana granulosa are continuous into the contents of the follicle (central substance). *c. s.* Central substance, very different from the liquor folliculi of the ordinarily-described Mammalian follicle, both in structure and in the fact that the ovum lies within it. The substance stains with extreme difficulty, and often not at all. Its external layer is rather darker, bordering on the lighter intermediate layer (but these effects are not due to the staining), while the membrana granulosa stains deeply. The structure and relations of these layers will appear in the more highly magnified figures. *F'*. Large follicles of similar structure to the one described above, but not cut through in the plane of the ovum. It is seen that as the follicles increase in size they project more and more from the surface. The contents of the follicles have contracted, thus leaving a space between the membrana granulosa and the follicular walls. In one case the membrana granulosa has been thrown into folds. This appearance is different from that seen in higher Mammalia, where the fluid contents of the follicle shrink away from the membrana granulosa (which rarely becomes detached from the follicular walls) in the process of hardening, and probably also drain away to some extent. Here in the large follicles the contents generally fill the space included by the membrana granulosa. *F'''*. Tangential sections through large follicles, in some

## DESCRIPTION OF PLATE V—continued.

cases only showing the fibrous tunic, in others the *membrana granulosa*; while in one instance the intermediate layer is also cut through and appears as a central light area surrounded by the darker *membrana granulosa*. *g.f.* Groups of small follicles, in some cases containing a larger follicle with the primary single-layered epithelium, advanced into the many-layered *membrana granulosa*. These small follicles form an irregular cortical layer of Schrön, external to this is the albuginea and germinal epithelium. Isolated follicles in various stages are also scattered through the cortical part of the ovary. When these are pressed by the rapid growth of a large follicle, they give way and become drawn out in the direction of the circumference of the latter (this is well seen in Fig. 4). *c. l.* Corpus luteum, filled with yellowish cells, among which the external tissues intrude, carrying blood-vessels with them. *V. P.* The very vascular central part of the organ (*zona vasculosa*). *g. c.* Lobules of granular cells, often arranged in elongated masses. These are doubtless the remains of the Wolffian body. Although especially present towards the central part of the organ, scattered lobules and masses also occur in the cortical part (Fig. 4).

The next five figures (2—6) show the gradual development of a follicle, the earliest stage being shown in the small follicles of Fig. 4. All the figures are magnified fifty diameters, so that the relative sizes express a true ratio.

FIG. 2.— $\times 50$ . A small follicle, with the included ovum. *m. g.* *Membrana granulosa*, with its external basement membrane. The shape of the cells is very difficult to determine: sometimes they appear polygonal, sometimes flattened, and sometimes there is an indefinite appearance of columnar structure in the external layer. The nuclei stain deeply. The indefinite shape of the cells is doubtless due to their extreme flexibility. Subsequent investigation has shown that the outer cells are columnar, the others polygonal. It is perfectly clear that there is no regular layer of columnar cells, with a radiate arrangement, round the ovum. In fact the layer next the ovum often seems to be composed of rather flattened cells. Here again is an important point in which the Marsupial follicle does not follow a very characteristic feature of the higher Mammalia. *o.* The ovum itself, the reference mark being placed in the vitellus. The *zona pellucida* (?) is excessively thin, and I could detect no trace of another layer. The substance of the vitellus appears as an extremely fine network (in this specimen hardened with chromic acid), which does not stain readily. *n.* The subcentral nucleus (germinal vesicle). This structure sometimes stains very slightly, or not at all (as shown in this figure); while in other cases it stains deeply (Fig. 3). After the methods employed in hardening, it shows a coarse network. It possesses a distinct limiting membrane. There is a distinct spherical nucleolus (germinal spot), which is clearly defined and generally very dark.

FIG. 3.— $\times 50$ . The reference marks are the same as those of the last fig. The nucleus in this case stains deeply, and it is placed at the circumference of the ovum.



## DESCRIPTION OF PLATE V—continued.

FIG. 4.— $\times 50$ . *g. f.* Groups of small follicles, with a single layered flattened epithelium. The nucleus of the ovum is central. That one of the group nearest to a large and growing follicle has become compressed into an oval shape, and this is still more the case with the members of two other small groups of follicles (*e. f.*). *g. c.* Granular cells, arranged in two elongated masses (remains of the Wolffian body). The reference marks describing the large follicle are in most cases similar to those previously explained. *V. C.* Vesicular cells, transitional into the central substance (*c. s.*). These cells are an addition to the intermediate layer (*i. l.*), and are not present in the largest follicles. They are swollen, unstained, with no distinct nucleus, and are doubtless in a state of transition into the central substance. The ovum (*o*) has no distinctly marked nucleus; it becomes indistinct in the increase from the size shown in Fig. 3 to that shown in this fig. I have never been able to detect it in the largest ovarian ova. There is a thin layer of the membrana granulosa cells surrounding the ovum as in the higher Mammalia, but the cells are not columnar. The central substance (*c. s.*) is finely granular and clot-like. It has shrunk away from the membrana granulosa. In both these respects it more resembles the appearances presented by the hardened liquor folliculi of higher Mammalia, and in both respects the resemblance is greater in younger follicles and gradually disappears as the follicles become older. The present figure would fairly represent a stage in the development of the Graafian follicle of higher Mammalia, except for the absence of columnar cells round the ovum. In this and the succeeding figs. 5—7 the outer cells of the granulosa should be columnar, the others more distinctly polygonal.

FIG. 5.— $\times 50$ . A larger follicle than the last. The reference marks are the same. There are still cells of the membrana granulosa round the ovum, but they have no connection with the peripheral cells, thus diverging from the higher Mammalian type. The central substance (*c. s.*) still remains finely granular, but it has not shrunk away from the membrana granulosa.

FIG. 6.— $\times 50$ . An apparently full-sized follicle. Most of the references are the same. The ovum (*o*) is now seen to be embedded in the central substance (*c. s.*) with no traces of cells round it (occasionally they can be detected). The central substance (after the use of the above-mentioned reagents) is a granular network, which stains with difficulty and does not shrink away from the membrana granulosa. The fibrous tunic (*f. t.*) is seen to be especially densely fibrous in its outer part, and vascular within. The membrana granulosa is irregularly thickened opposite to the ovum, and at this point the fibrous tunic thins away so that the follicle nearly penetrates it. This fact and the slight thickness which separates the follicle from the external surface, together with the convexity of the latter, make it probable that the follicle is ripe. The ovum is always placed (as far as I have observed) on that side of the ripe follicle which is directed towards the external surface of the ovary, though not always at the most convex point. The granulosa cells

## DESCRIPTION OF PLATE V—continued.

should be more distinct: they are drawn as they appeared after the action of chromic acid.

FIG. 7.— $\times 188$ . A portion of the periphery of a large follicle (about 2 mm. in diameter), showing the details of some structures already mentioned. The reference figures have been already explained. The membrana granulosa is relatively thin, and forms an even layer. The intermediate layer (*i. l.*) is seen to form a coarse network, the origin of which from the cells of the membrana granulosa is proved by actual continuity and by the presence of nuclei at the nodal points. Nuclei are sometimes met with at a considerable distance from the membrana granulosa. The central substance (*c. s.*) is continuous with the strands of the intermediate layer. It forms a very granular network, with thick irregular strands. These are the appearances seen after the action of hardening reagents. The outermost cells of the granulosa should be columnar.

FIG. 8.— $\times 9$ . A transverse section of the ovary of Ornithorhynchus. The whole organ was much contracted and altered. *st.* Stroma, very much contracted, and of which it was impossible to make out the true structure. In the central part of the section there are a few small blood-vessels. The ovary is a flattened oval body, and it is possible that blood-vessels are more abundant in the central part of some other region than that seen in this section, and it is likely that here they are much concealed by the contracted state of the tissues. The follicles (*F.*) are very abundant all round the periphery of the section, and the large ones project from the surface. There is also a tendency towards constriction of the larger follicles, thus resembling the arrangement in some other vertebrates where the constriction is carried so far as to produce a stalked appearance. It is also probable that the constriction is far more marked in a fresh ovary than in the one from which this section is taken. The light spherical spaces inside the follicles represent the ova, which are thus much larger than in other Mammalia, although the follicles are far smaller than those of Phalangista. The line enclosing the light spaces represents the zona pellucida (?) and the single-layered follicular epithelium. Outside this is the basement membrane of the epithelium and an external layer, which represents the fibrous tunic. *c. l.* Structures representing the Corpora lutea. There seem to be different forms of these. In some few cases I have found the follicular lumen apparently filled with yellowish cells, and the fibrous tunic thickened. In other cases, evidently soon after the escape of the ovum, the lumen was partially occupied by a yellow shrunken clot, which had also stained the adjacent tissues. Most commonly, however, the lumen seems to be obliterated by a gradual thickening of the tunic, in which vessels can be detected. Some of those in the section are of this kind, while others still show a slight trace of the lumen. It is of course probable that some of the appearances mentioned (if not all) are different stages of one history, but these apparently highly peculiar structures can only be satisfactorily

## DESCRIPTION OF PLATE V—continued.

made out when better material is obtained. It is seen that these structures do not project from the surface to the same extent as the large follicles.

FIG. 9.— $\times 188$ . A small follicle from the ovary of *Ornithorhynchus*. At this stage the follicle is not at all unlike those of the higher *Mammalia*, but in one of this size the follicular epithelium (see Fig. 4, *g.f.*) would be flattened, and the zona pellucida less marked. *f.e.* Follicular epithelium composed of cubical cells, resting upon a basement membrane. *z.p.* Structureless membrane probably the zona pellucida, generally strongly adherent to the follicular epithelium. *o.* Ovum, with *n.* the nucleus, containing a probably altered nucleolus.

FIG. 10.— $\times 188$ . A rather larger follicle. The references are similar, except that *f.t.*, the fibrous tunic, is added in this case.

FIG. 11.— $\times 188$ . The ovum in this instance had shrunk, but retained its shape notwithstanding. The follicle was about twice the diameter of the ovum (and the latter when unshrunk must have filled it), and the interval was filled with the debris of epithelium and zona pellucida. Two of the fragments are drawn. The difference in size between the cells of this figure and the preceding may be due to different degrees of contraction. The reference letters are the same as those of the preceding figures. The nucleus (*n*) is seen to possess a distinct limiting membrane, within which it has shrunk. The external part of the ovum (*o*) is finely granular, even under high powers; within this is a zone chiefly composed of large granular spheres, and again in the centre the ovum becomes finely granular. The nucleus is situated in the intermediate zone, and is probably on its way to the periphery. Although the ova of the specimen here described were very much altered and sometimes completely disintegrated, yet I found the appearances so often and so distinctly that I think it very probable that they represent to a large extent the actual condition of the ovum.

FIG. 12.— $\times 405$ . The periphery of a large follicle (probably 1 mm. in diameter, and more when uncontracted) highly magnified. *f.t.* Fibrous tunic, from which the epithelium (*f.e.*) and its basement membrane (*b.m.*) have shrunk away. *z.p.* Zona pellucida, on the inner surface of which is a thin layer (*z'.p'.*), which may be the indication of a laminated structure, or may be possibly an optical effect or a result of change. In some cases I have been able to detect two such lines. *v.m.* A fine layer within the zona pellucida. *e.l.* External layer of the vitellus, which stains deeply and is finely granular. This is very constant, and adheres closely to the zona pellucida, so that it is common to find short arcs composed of these two layers with the follicular epithelium also attached to the outside of the zona pellucida, while the rest of the ovum has disintegrated and the periphery has thus been broken. *g.s.* Large finely granular spherical or oval masses. Between these and the deeply-staining external layer (*e.l.*), just described, there is a finely granular layer which does not stain deeply.

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### EXPLANATION OF PLATES VI, VII, VIII, IX, X, XI, & XII,

Illustrating Professor Lankester's Memoir on the "Skeleto-  
trophic Tissues and Coxal Glands of *Limulus*, *Scorpio*,  
and *Mygale*."

#### PLATE VI.

FIG. 1.—Entosternite or prosomatic plastron of *Limulus*, dorsal surface. *Ph. N.* Pharyngeal notch, where the pharynx is embraced by the entosternite. *L. A. P.* Left anterior process or cornu. *R. A. P.* Right anterior cornu. *A. L. R.* Anterior lateral rod or tendon (paired). *P. L. R.* Posterior lateral rod or tendon (paired). *P. L. P.* Posterior lateral process or cornu (paired). The bifurcate median posterior process is not lettered. Natural size.

FIG. 2.—View of ventral surface of the same. *N. F.* Neural fossa, in which the great prosomatic ganglia lie. *P. M. P.* Posterior median process. *P. V. P.* Posterior ventral process. Other letters as in Fig. 1. Natural size.

FIG. 3.—Ventral view of the entosternite of *Mygale* sp. *Ph. N.* Pharyngeal notch. Magnified seven diameters.

FIG. 4.—Dorsal view of the entosternite of *Mygale*. *Ph. N.* Pharyngeal notch. The three pairs of upstanding rod-like processes correspond to the two pairs of lateral rod-like processes, *A. L. R.*, *P. L. R.*, of *Limulus*. Observe also the close similarity of the posterior median process in *Mygale* and *Limulus*. Magnified seven diameters.

FIG. 5.—Dorsal view of the entosternite of a scorpion (*Buthus cyaneus* of Ceylon). *a. p.* Anterior process (paired). *l. m. p.* Lateral median process (paired). *pp.* Posterior lateral process (paired). *p. f.* Posterior flap, a wide-spreading diaphragm-like outgrowth from the posterior lateral processes. *G. C.* Gastric canal. *A. C.* Arterial canal. *D. R.* Dorsal ridge (paired). *Snp.* subneural arch. *asp.* Anterior process (paired) of the subneural arch. *m*<sup>1</sup>. Anterior muscular perforation (paired) of the posterior flap. *m*<sup>2</sup>. Posterior muscular perforation (paired) of the posterior flap. Magnified ten diameters.

FIG. 6.—Ventral view of the entosternite of a scorpion (*Buthus cyaneus* of Ceylon). *R. A. P.* Right anterior process. *L. A. P.* Left anterior process. *l. m. p.* Lateral median process (paired). *p. p.* Posterior process (paired). *p. f.* Posterior flap. *Snp.* Subneural arch. *asp.* Anterior process



EXPLANATION OF PLATES VI, VII, VIII, IX, X, XI, XII—continued.

(paired) of the subneural arch. *N. C.* Neural canal. *G. C.* Gastric canal. *A. C.* Arterial canal. *m*<sup>1</sup> Anterior, *m*<sup>2</sup> posterior muscular perforations of the posterior flap. Magnified ten diameters.

FIG. 7.—Lateral view of the entosternite of a scorpion (*Buthus cyaneus* of Ceylon). Letters as in Fig. 5. Magnified ten diameters.

PLATE VII.

FIG. 1.—Section of a portion of the entosternite of a scorpion (*Androctonus funestus*). Magnified 300 diameters. The preparation has been stained with borax-carmin, which colours the nuclei of the cells and *also*, but less deeply, the dense fibroid matrix, leaving the protoplasm of the cells almost colourless. *a.* Muscular fibre attached to the entosternal tissue by its sarcolemma. *b.* Protoplasmic corpuscles of the lacunar connective tissue adjacent to the entosternite. *c.* Lacuna or central space of an areole of the lacunar connective tissue.

FIG. 2. Another piece of the same, in order to show transition from the "fibro-massive" to the "lacunar" variety of connective tissue. *c.* Lacuna or central space of an areole of the lacunar connective tissue.

FIG. 3.—Section of a portion of the entosternite of the King-crab (*Limulus polyphemus*, Latr.). The nuclei of the cells and the surrounding matrix have taken the stain of borax-carmin, but the protoplasm of the cells is unstained. Magnified 300 diameters.

FIG. 4.—Section of a portion of the entosternite of a bird's-nesting spider (*Mygale* sp.). The same treatment has been adopted and the same result obtained as in the specimens Figs. 1 and 2.

PLATE VIII.

FIG. 1.—Section of a portion of the entosternite of *Apus canceriformis*, showing a structure identical with that of *Scorpio* and *Limulus* figured in the preceding plate. *a.* Nuclei of the cells. *p.* Unstained protoplasm of the cells. *c.* Dense skeletal matrix. Magnified 300 diameters.

FIG. 2.—Dorsal view of the entosternite of *Apus canceriformis*. Magnified twenty-five diameters.

FIG. 3.—Ventral view of the same.

FIG. 4.—Blood-corpuscles of *Limulus*. Magnified 1000 diameters.

FIG. 5.—Blood-corpuscles of *Buthus cyaneus*. *a b c d*, living; *e f*, after the addition of acetic acid. *g.* Nucleus of the same more highly magnified. Excepting *g*, all are magnified 1000 diameters.

FIG. 6.—Blood-corpuscles of a South African *Androctonus*. Drawn from freshly-shed blood.

## EXPLANATION OF PLATES VI, VII, VIII, IX, X, XI, XII—continued.

FIG. 7.—Transverse section of the coxal gland of a young specimen of *Scorpio* (*Euscorpius*) *Italicus*, Roes. Magnified about eighty diameters. *N.* Core or medullary tissue. *cbv.* Axial blood-vessel traversing the core of medullary tissue. *K.* Lumen of the cæca of the gland. *o.* Inter-cæcal space or channels (partly occupied by very delicate connective tissue, see Fig. 5, Plate XII). *L.* Gland-epithelium. *F.* Cæca of the gastric gland. *x.* Membranous connective tissue enclosing the gland. *y.* Blood-space.

FIG. 8.—Section of a portion of the antennary coxal gland (green-gland) of *Palæmon*. *K.* Lumen of the gland-cæca. *L.* Gland-epithelium. *m.* Inter-cæcal connective tissue. Magnified 820 diameters.

## PLATE IX.

FIG. 1.—Section of a portion of the entosternite of *Limulus*, taken from the central region, deeply. It shows the development in this part of the entosternite's tissue of a well-defined system of branched anastomotic fibres (presumably elastic) which traverse the homogeneous matrix, and gives this variety of the tissue a close resemblance to the yellow elastic cartilage of *Mammalia*. *a.* Cell-nuclei. *b.* Branched elastic fibres. *c.* Homogeneous matrix. Magnified 300 diameters.

FIG. 2.—Section of a portion of one of the tendinous lateral rods of the entosternite of *Limulus* (*A. L. R.* of Plate VI, Figs. 1 and 2), taken parallel with the length of the rod in order to show the regular arrangement of the cells in rows, resembling white fibrous cartilage. *a.* cell-nuclei. *c.* Matrix. Magnified 300 diameters.

FIG. 3.—Fragment of a membranous ligament of the heart of *Limulus*, stained and magnified 300 diameters. Above the dotted line *xy*, the surface of the membrane is in focus in order to show the closely set covering of protoplasmic corpuscles, indicated by their oblong well-stained nuclei (*a*). Behind the line *xy*, a deeper stratum is represented, consisting of undulating and matted fibres. This is representative of the structure of "membranous connective tissue" in *Arachnida*.

FIG. 4.—Portion of a tendon of the branchio-thoracic muscle of *Limulus*, being the connective-tissue investment of one of the hollow tegumentary ingrowths corresponding to the parabranchial stigmata. The specimen is selected to show the fine parallel fibrillation of the matrix and the absence over large areas of any protoplasmic cells. *a.* Cell-nuclei. Magnified 300 diameters.

## PLATE X.

FIG. 1.—Section through lacunar tissue (left side of the drawing) and the cortical substance of a lobe of the coxal gland (right side) of *Limulus*. Magnified 275 diameters. *a.* Nuclei of the cells of the lacunar connective

## EXPLANATION OF PLATES VI, VII, VIII, IX, X, XI, XII—continued.

tissue. *b.* Skeletal product (capsule-like trabeculæ) of the lacunar connective tissue. *d.* Spherical refringent bodies lying in the protoplasm of the cells of the lacunar connective tissue. *e.* Central lacuna or space of an areole of the lacunar connective tissue. *F.* A cæcum of the gastric gland (so-called liver) in transverse section. *G.* A testicular ampulla. *H.* Line of transition from the lacunar connective tissue to the compact many-celled connective tissue forming the cortical substance of the coxal gland. *I.* Compact connective tissue forming the cortical substance of the coxal gland, containing some spaces (*e*) which, though resembling the gland-cæca, are identical in nature with the lacunæ of the lacunar connective tissue. *K.* Lumen of cæca of the coxal gland. *l.* Gland-epithelium of the coxal gland lining the spaces *K.* *m.* Intercæcal connective tissue or framework of the coxal gland.

FIG. 2.—A portion of the lacunar tissue of *Limulus* with five gastric cæca in section (*FF*). *G.* Testicular ampulla. Magnified 150 diameters.

FIG. 3.—A portion of the lacunar tissue of a scorpion (*Androctonus funestus*) with several gastric cæca in section (*FF*), for comparison with FIG. 2. Magnified 150 diameters.

## PLATE XI.

FIG. 1.—Lacunar tissue of *Limulus*, magnified 430 diameters. *a.* Nuclei. *b.* Skeletal trabecular element. *d.* Refringent corpuscles. *e.* Central space or lacuna of an areole. *p.* Protoplasm of the cells.

FIG. 2.—A similar specimen with less abundant protoplasm, showing also fine blood-vessels (*B. V.*) and contained blood-corpuscles (*b. c.*). Other letters as in FIG. 1. Magnified 430 diameters.

FIG. 3.—Lacunar tissue of a scorpion (*Androctonus funestus*), coloured and lettered in the same way as Figs. 1 and 2. Magnified 430 diameters.

FIG. 4.—Capsuligenous (cartilaginoid) tissue from the entapophysial ligament of *Limulus*. Magnified 300 diameters. The transition of fibroid tissue (similar to that of the entosternite, and here forming the cortical layer of the ligament) into the capsuligenous tissue is shown. *a.* Cell-nuclei. *b.* Capsule-wall. *e.* Space within the capsule not occupied by protoplasm, corresponding to the space *e* in Figs. 1 and 2. *x.* Problematic enlargements of the skeletal substance, possibly blood spaces. *R.* Fibroid cortical tissue of the ligament. *a a.* Cell-nuclei of the same. *b b.* Matrix (skeletal substance) of the same, continuous with the capsules of the capsuligenous tissue.

FIG. 5.—Section of a portion of the coxal gland of *Limulus*, in order to show the irregular form of the spaces (really anastomosing with one another) which constitute the lumen of the gland *K.* Magnified about eighty diameters.

FIG. 6.—Gland-epithelium layer of the coxal gland of *Limulus*, as seen in

# EXPLANATION OF PLATES VI, VII, VIII, IX, X, XI, XII—continued.

the fresh condition, the red granules *g* which give the gland its brick-red colour being here represented with their natural tint. *f*. Free border of the epithelium. *x*. Deep border. Magnified 2500 diameters.

FIG. 7.—A somewhat thick section of the capsuligenous tissue of *Limulus*, with the protoplasm omitted in order to show the form and connection of the capsules. Magnified 300 diameters.

FIG. 8.—A similar view of the skeletal element (membranous capsules or trabeculæ) of the lacunar tissue of *Limulus*.

FIGS. 9 and 10.—Large-celled connective tissue of scorpion (*Androctonus funestus*). *b*. Skeletal trabeculæ. Magnified 430 diameters.

## PLATE XII.

All the figures are drawn to the same scale, viz. they represent the object magnified 500 diameters. The colours are arbitrary.

FIG. 1.—Portion of the coxal gland of *Mygale* sp. *lp*. Protoplasm of the gland-epithelium cells. *ln*. Nuclei of the gland-epithelium cells. *cr*. Striated cortical layer of the gland-epithelium cells. *col*. Colloid substance of the inter-cæcal tissue or framework. *cn*. Nuclei of the same tissue stained by borax-carminé, the surrounding protoplasm unstained. *K*. Lumen of the glandular cæca.

FIG. 2.—Tangential section of a cæcum of the coxal gland of a scorpion (*Buthus cyaneus*), showing the striated cortical layer surrounding the gland-epithelium cells. Letters as in Fig. 1.

FIG. 3.—Portion of the entosternite of *Mygale*, in which there are fissures containing masses of colloid substance (*col*). The matrix (*c*) of the entosternite is coloured grey, the nuclei (*cn*) of the cells are red, and the surrounding protoplasm is unstained.

FIG. 4.—Portion of the coxal gland of *Limulus*. *a*. Nuclei of the inter-cæcal connective tissue. *b*. Fibres of the same. *e*. Lacunæ of the same. Other letters as in Fig. 1.

FIG. 5.—Portion of the coxal gland of a scorpion (*Buthus cyaneus* of Ceylon). *Kc*. Coagulum in the lumen of one of the glandular cæca. *O*. Space or channel of the inter-cæcal connective tissue (corresponding to *e* in Fig. 4). *m*. Nuclei of the inter-cæcal connective tissue (corresponding to *a* in Fig. 4). *cr*. Cortical striated substance of the glandular epithelium. *ln*. Nuclei of the gland-cells. *lp*. Protoplasm of the gland-cells.



## On the Chlorophyll Corpuscles of some Infusoria.

By

**Jessie A. Sallitt.**

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With Plates XIII and XIV.

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A FORM of chlorophyll corpuscle present in *Spongilla* and *Hydra viridis*, which is peculiar to animals, has been recently described in this Journal by Professor Lankester.<sup>1</sup> In connection with this work, I have at his suggestion examined several green forms of Infusoria, and I wish to thank him here for the help he has most kindly given me.

The animals I have examined are species of *Paramecia*, *Stentor*, *Vaginicola*, *Vorticella*, *Phacus* and *Euglena*.

The chlorophyll corpuscles in most of these are so much alike, that a description of each would be in great part repetition, so I describe fully only those of one, that is *Paramecium bursaria*. The figures given of the rest sufficiently show their similarity to the typical form.

In *Paramecium bursaria* and *Stentor polymorphus* the corpuscles are large and clearly defined, and I have been able to follow their mode of division. The corpuscles of the remaining forms, from the few stages observed, evidently divide in the same way.

*Paramecium bursaria*.—The chlorophyll corpuscles are very numerous, and are scattered through the endoplasm of the animal. They are usually spherical. They vary in size

<sup>1</sup> E. Ray Lankester, "Chlorophyll Corpuscles and Amyloid Deposits of *Spongilla* and *Hydra*," 'Quarterly Journal of Microscopical Science,' 1882.

from .0025 mm. to .006 mm. in diameter. Each corpuscle consists of two parts: 1, a ball of clear protoplasm; 2, an investing cup-like layer of chlorophyll-containing protoplasm (fig. 1). For convenience I call the chlorophyll-containing layer chloroplasm. The chloroplasm is of a bright light green colour. It is bleached by alcohol, and is then seen to consist of granular protoplasm. With iodine the whole corpuscle is stained brown; the chloroplasm stains more deeply than the central protoplasmic mass.

Division of the corpuscles.—Simple division (fig. 2) begins by cleavage of the chloroplasm at right angles to the margin of the cup. The division gradually extends down to the base of the cup, and at the same time the chloroplasm frequently grows upwards so as to enclose more completely the central ball. This is followed by cleavage of the central protoplasm which completes the division. In many cases division is preceded by elongation of the corpuscles in a direction parallel to the mouth of the cup, and the cleavage of the clear protoplasm is coincident with that of the chloroplasm (fig. 2, *a*).

Division in three parts occurs by a second division in one half of a dividing corpuscle (fig. 3). The fresh cleavage line extends as in the first case down to the chlorophyll pole of the corpuscle.

Division in four parts only differs from that in three by the occurrence of a second division in each half of the dividing corpuscle. The chloroplasm grows up to surround the protoplasmic ball before division is effected in the latter. In consequence of this, when the division is complete in the chloroplasm, the corpuscle appears to be dividing by simultaneous horizontal and vertical cleavage (fig. 4). An earlier stage shows that both divisions are vertical.

The newly formed corpuscles are usually small and somewhat angular (fig. 5). They become more spherical as they grow larger.

*Stentor polymorphus* (figs. 6—13).—The corpuscles closely resemble in form, mode of division and intensity of colour, the corpuscles of *Paramecium bursaria*. They are slightly

larger. They vary in size from  $\cdot 0025$  mm. to  $\cdot 0075$  mm. in diameter. They are present in great numbers throughout the endoplasm, and circulate in the streaming protoplasm.

*Vaginicola grandis*.—The corpuscles are fairly numerous. They are scattered in the endoplasm. In size, form, colouring and division they resemble those of *Paramecium bursaria* (fig. 14).

*Vorticella chlorostigma*.—No corpuscles are present. The chlorophyll is apparently diffused through the endoplasm which is very transparent.<sup>1</sup> Its colour is a very delicate green. It appears loosely held by the protoplasm; slight pressure causes the colour to disappear. It is quickly dissolved by alcohol.

In another *Vorticella* which resembles *Vorticella campanula*, but which I have been unable to identify, I have found corpuscles of the typical form. They are numerous and circulate in the endoplasm. The chlorophyll is bright green.

*Phacus*.—I have examined *Phacus triqueter*, *Phacus longicaudis*, and *Phacus glabra*.<sup>2</sup>

In each of these forms the chlorophyll is contained in corpuscles which agree in general structure with those of the above-mentioned Ciliata. They are, however, more fragile, and the colour is a very pale green. They vary in size from  $\cdot 0025$  mm. to  $\cdot 0075$  mm. in diameter. The separate corpuscles are readily seen in *Phacus triqueter* and *Phacus longicaudis* lying beneath the cuticle (fig. 15). In *Phacus glabra* they are usually quite obscured by several large amylaceous bodies, and the chlorophyll appears diffused until the corpuscles are set free by pressure.

*Euglena*.—In *Euglena acus* and *Euglena oxyuris* (figs. 19—21) the chlorophyll corpuscles are numerous. They

<sup>1</sup> Professor Engelmann, of Utrecht, has also recently described a *Vorticella* in which the chlorophyll is diffused throughout the protoplasm, and is not localized in corpuscles. This mode of occurrence of animal chlorophyll once and for all disposes of Brandt's assumption that chlorophyll in animals is always due to parasitic Algæ.—E. R. L.

<sup>2</sup> A recently discovered *Phacus*, sent to me by Mr. Bolton.

closely resemble those of *Phacus*. The colour of those of *Euglena oxyuris* is a slightly deeper green. The corpuscles are from  $\cdot 0025$  to  $\cdot 005$  mm. in diameter in *Euglena acus*, and from  $\cdot 0025$  to  $\cdot 007$  mm. in diameter in *Euglena oxyuris*.<sup>1</sup>

In *Euglena viridis* the corpuscles agree in structure with the typical form; they consist of a colourless and a green part. Their shape is, however, considerably modified; they are much flattened, and are irregular in outline. They are not numerous, and frequently in young forms only two or three are present. Professor Lankester informs me that the *Euglenæ* produced by division contain at first only one lenticular corpuscle. They lie usually close under the cuticle with their green surface adhering to it, as in fig. 22, *a*, *c*. In their normal position the colourless layer is only seen when the corpuscles are dividing. It is best seen when the free corpuscles are viewed from the side. The corpuscles vary considerably in size; the longer axis is from  $\cdot 005$  mm. to  $\cdot 009$  mm. in length, and the shorter axis about  $\cdot 0025$  mm. The chlorophyll is an intense green; frequently in young forms a red colour is also present. In many cases the chlorophyll appears diffused through the endoplasm. Mr. Saville Kent considers that the chlorophyll in this form is normally diffused. He says:—"The brilliant green hue of the entire subcuticular parenchyma affords perhaps the most pre-eminent example of such diffuse colouration."<sup>2</sup> He regards the chlorophyll bodies as the result of the splitting up of the "entire coloured inner substance" previous to multiple division. I have not followed the process of multiplication in the *Euglena*, but several things seem to oppose this view. In many cases where the chlorophyll appears, diffused careful examination shows more or less distinctly the

<sup>1</sup> The lateral compression of the body in *Euglena acus* and *Euglena oxyuris*, its slight flexibility, and the total absence of the simple muscular movement characteristic of the *Euglenidæ*, as well as the form of the corpuscles, seem to show that these animals are more closely connected with the *Phacidæ* than with the group in which they are now placed.

<sup>2</sup> W. Saville Kent, 'Manual of Infusoria.'



margins of the corpuscles, in part hidden from view by numerous amylaceous bodies; and I have found, with careful pressure, corpuscles passing out from the endoplasm. This might be due to the fact that division was already beginning in the protoplasm; but the corpuscles cannot result from the breaking up of the entire coloured endoplasm, because when formed they are surrounded by colourless granular protoplasm. At most the chlorophyll could only be diffused in the layer immediately beneath the cuticle. The fact that the corpuscles agree in structure with the typical form, and that they are present in young *Euglenæ*, seems to make it improbable that they are formed as a preliminary to division, though they may originally have been so formed.

The corpuscles in all these animals have the same structure as those of *Spongilla* and *Hydra viridis*. The existence of a colourless and green layer appears characteristic of the animal chlorophyll corpuscle.

If the function of the chlorophyll in animals is the same as that ascribed to it in plants by Professor Pringsheim, the disposition of the chlorophyll in the animal corpuscle is better adapted to shelter the central colourless protoplasm than that of the substance of the cell. So the greater saving of oxidisable material should take place in the corpuscle itself. I have tested the animals examined with iodine, but have found no trace of starch in the corpuscle or in the endoplasm. Bright transparent bodies are not infrequently present in the corpuscles, but I have not ascertained their nature.

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## DESCRIPTION OF PLATES XIII and XIV

Illustrating Miss Jessie A. Sallitt's Paper on "The Chlorophyll. Corpuscles of Infusoria."

FIGS. 1 to 5.—Chlorophyll corpuscles of *Paramecium bursaria*.

1.—Group of corpuscles.

2.—Corpuscles dividing. *a*. Series showing gradual division.

*b*, *c*, *d*. Different views of the same corpuscles:

3.—Corpuscles dividing into three.

4.—Corpuscles dividing into four.

5.—Recently freed corpuscles.

FIGS. 6 to 13.—Chlorophyll corpuscles of *Stentor polymorphus*.

6.—Group of corpuscles.

7.—Corpuscles dividing. *a. b. c.* Different views of the same corpuscles, showing the chloroplasm to be a superficial layer enclosing a ball of transparent protoplasm.

8.—Corpuscles dividing into three. *a.* Shows the chlorophyll pole. *b.* The protoplasmic pole. *c.* Lateral view. *d.* Advanced stage in division.

9.—Corpuscles dividing into four. *a.* Division of chloroplasm. *b.* Chlorophyll and protoplasmic poles of a corpuscle. *c.* Lateral view. *d.* Views of a rotating corpuscle. *e.* Advanced stage in division.

10.—Irregularly dividing corpuscle.

11.—Recently freed corpuscles.

12.—Corpuscles bleached by alcohol, granular and spongy-looking.

13.—Bleached corpuscles stained by iodine.

FIG. 14.—Corpuscles of *Vaginicola grandis*.

FIG. 15.—*Phacus longicaudis*, showing separate corpuscles (F. 1)<sup>1</sup>.

FIG. 16.—Corpuscles of *Phacus longicaudis*.

FIG. 17.—*Phacus glabra* (F. 4), chlorophyll corpuscles obscured by amylaceous bodies, *a, a.*

FIG. 18.—Corpuscles of *Phacus glabra*.

FIG. 19.—Portion of *Euglena acus*, showing chlorophyll corpuscles (F. 4).

FIG. 20.—Corpuscles of *Euglena acus*.

FIG. 21.—Corpuscles of *Euglena oxyuris*.

FIG. 22.—*a, b, c.* *Euglena viridis*, showing separate corpuscles. *a.* Motile forms (F. 1). *b, c.* Encysted forms (D. 4).

FIGS. 23, 24.—Corpuscles of *Euglena viridis*.

FIGS. 25, 26.—*a, b, c.* Different views of the same corpuscles.

FIG. 27.—*Euglena*, showing corpuscles and colourless protoplasm passing out from the apparently green endoplasm (F. 4).

FIG. 28.—Young *Euglenæ* (F. 4).

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<sup>1</sup> Zeiss's powers.

On the Head Kidney of *Bdellostoma*, with a  
Suggestion as to the Origin of the Supra-  
renal Bodies.

By

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With Plate XV.

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THE structure of the greater part of the kidney of *Bdellostoma* and the Myxinoids generally has been known since the time of Johannes Müller. It consists of a simple segmental duct on each side, which represents both the Wolffian and Müllerian ducts of the higher Vertebrates, giving off in its course a series of short tubules, each of which ends in a large glomerulus. These tubules are segmentally arranged, a single pair being present in every segment of the body in the anterior three fourths of the region lying between the anus and the hinder border of the pericardium. The relations of this system of simple segmental tubules, opening into a segmental duct, were described with perfect accuracy by Johannes Müller;<sup>1</sup> and no important additions have since been made to the account which he gave.

The whole system obviously represents the Wolffian body of the higher Vertebrates before the splitting of the segmental duct into Wolffian and Müllerian ducts.

In front of the kidney proper, however, there is on each

<sup>1</sup> 'Vergleichende Anatomie d. Myxinoiden,' Berlin, 1836—1845.

side a small, lobulated, apparently glandular body, whose structure is not so well known.

Each of these bodies is, in *Bdellostoma Forsteri*, from 20 to 25 millimètres long, and from 5 to 7 mm. broad; looked at with a simple lens, or with the naked eye, it is seen to consist of several small lobuli, which project into the cavity of the pericardium on the one hand, the whole gland being connected on the other with the connective-tissue adventitia of a great vein—the gland of the right side with the adventitia of the portal vein, that of the left with the anterior cardinal. In Pl. XV, fig. 1, an attempt has been made to represent the appearances seen on looking at the gland from the interior of the pericardium.

Johannes Müller<sup>1</sup> described these organs as consisting of "very small elongated lobuli, which are attached to blood-vessels, and are united with one another by loose connective-tissue. Each lobulus or cylinder consists . . . of a double row of cylindrical nucleated cells, like those of a columnar epithelium, the two rows of cells fusing with one another at the base of each lobulus. Between them run blood-vessels."

No further statements on the subject were published till 1875, when Prof. Wilhelm Müller<sup>2</sup> gave an account of some observations made on *Myxine glutinosa*. In this animal Prof. Müller found that the bodies in question were connected each with the segmental duct of its own side, while the "double rows of cells" of Johannes Müller he found to be segmental tubules of a perfectly normal character, communicating by ciliated funnel-shaped openings with the pericardium, and provided with glomeruli.

The obvious inference was that these organs represented that anterior part of the kidney which is so well developed in many larval Icthyopsida, and which is known as the "pronephros," or "head kidney."

Professor Müller, however, expressly states in his paper that his investigations were made upon very young animals.

<sup>1</sup> Loc. cit., part iii, pp. 7, 8.

<sup>2</sup> 'Jenaische Zeitschrift,' ix, 1875, pp. 111—113.



During the summer of last year, Mr. Sedgwick, while visiting the Cape of Good Hope, collected amongst other things a large number of very fine specimens of *Bdellostoma Forsteri*, var. *hexatrema*; and on his return to Cambridge he very kindly obtained permission from the Royal Society, for whom the specimens were collected, to allow me to examine their renal organs. On making a superficial examination of the so-called head kidneys, it was evident, as shown in fig. 1, that they were separated by a considerable distance from the anterior end of the segmental duct (*s. d.*), the only structures passing from one organ to the other being apparently blood-vessels. The subsequent preparation of a complete series of sections, the first of which passed through the anterior extremity of the "head kidney," the last through the beginning of the segmental duct, proved conclusively that with the exception of a rudiment to be spoken of presently, no trace of connection existed between the two organs.

Transverse sections showed the presence of a number of branched ducts, evidently the "pronephric tubules" of Wilhelm Müller, which opened on the one hand into the pericardium, and on the other into a central duct (figs. 3, 7).

These tubules (Pl. XV, fig. 7) had an average diameter of 6 mm.; there was no increase, but rather a diminution in diameter at the openings into the pericardium (figs. 2, 3, 7, *f.*). Each tubule was lined by cubical or columnar cells, the protoplasm of which was finely granular; each cell contained a large, elliptical, highly refracting nucleus, containing numerous coarse dark granules (fig. 7). At the mouth of each tube the columnar lining epithelium was continued into the flat pericardial epithelium (fig. 7, *p. c.*). No traces of cilia were found on the cells bounding the openings into the pericardium. Outside the lining epithelium was a well-marked basement membrane (fig. 7, *b. m.*); and outside this, in the spaces between the tubules, was a small quantity of connective tissue, and an exceedingly rich plexus of blood-capillaries (fig. 7, *b. c.*); so that during life a very considerable quantity of blood must be constantly passing between the tubules of the gland.

The tubules are for the most part aggregated into considerable lobuli; but here and there these lobuli become smaller, and in some sections tubules are seen which project separately into the pericardium; several such were cut transversely in the section from which fig. 3 was drawn, and they are seen to be separately invested by pericardium.

Passing inwards towards the centre of the gland, the tubules unite with one another, still maintaining the same characters, and not showing any appreciable change in diameter, till they finally open into a large central duct (fig. 3, c. d.).

The central duct is elliptical in cross section, its long diameter being about 0.2 mm., its short diameter 0.5 mm.; it may be single, as represented in the diagrammatic longitudinal section (fig. 2), or it may be divided into two or three anastomosing branches. It is lined by a single layer of very long and slender columnar cells, each about 0.07 mm. long by about 0.009 mm. broad, and having a large oval nucleus, with a dark outline and granular contents towards its outer end. The protoplasm of these cells is crowded with granules (fig. 4), and the free extremity of each is produced into a number of fine pseudopodia, round which are collected numerous granules (fig. 4). It is difficult to avoid the belief that the appearances described are due to the fact of the epithelium cells being actively amoeboid during life, and of their pouring into the lumen of the central duct a quantity of secretion granules.

Outside this epithelium is a strong basement membrane (figs. 4, 6, *m*), which is connected with a tolerably compact coating of connective tissue, investing the whole duct.

The lumen of the duct was filled, in all my preparations, with a larger or smaller amount of material resembling a blood-clot, and consisting of a finely granular matrix (fig. 5), in which were contained oval nucleated cells, identical, so far as I was able to see, with the red blood-corpuscles found in blood-clots from the surrounding vessels. After a careful comparison, both with sections and with teased-out preparations of the blood-clots of the great veins, I have been unable to come to any other conclusion than that the central duct does actually con-

tain in preserved specimens a blood-clot, and, therefore, in the living animal blood.

The above description applies to the main body of the duct; anteriorly it gives off a bunch of tubules, similar in all respects to those given off from its sides (see diagram, fig. 2), while posteriorly it ends in a mass of tissue (figs. 2 and 6 and 7), resembling the trabecular supporting tissue of a lymphatic gland. Fig 6 shows a portion of the periphery of a section through this tissue. It is seen to consist of a network of nucleated, branched connective-tissue cells, with elongated meshes, in which are several scattered blood-corpuscles (*b. c.*).

This lymphatic tissue is covered by a well-marked epithelium (*b*), forming the capsule of a large glomerulus (figs. 2 and 6, *gl.*), which lies close to it. From this glomerulus strands of blood-vessels pass off at frequent intervals into the lymphatic tissue. Such a strand is figured at *x* in fig. 6.

Owing to the impossibility of injecting a capillary plexus in an animal which has been preserved in chromic acid, I have not been able to obtain any very definite proof that blood can pass by these strands of vessels into the lymphatic tissue of the duct, and so into its lumen; but I am strongly inclined to believe that this is the case.

I have occasionally seen capillaries leading directly from the lumen of the central duct, though I have been unable to follow them for any distance.

Until, however, further observations on fresh specimens can be made, I venture to think that I have shown tolerably good reason for assuming that the blood enters the lumen of the central duct of the "head kidney" through the glomerulus at its posterior extremity.

In some of my series of sections there is a considerable interval between the glomerulus just described and the segmental duct, which is occupied by nothing but connective tissue. In other, presumably younger specimens, I find traces of a continuation of the renal duct into the head kidney; though in no case have I seen a continuous lumen in the connecting piece.

It therefore seems to me that the organ, whose anatomy I have just described, may very probably have resembled, in its earlier stages, the head kidney described by Wilhelm Müller, in which case it would have to be regarded as a part of the embryonic kidney, modified, in connection with the needs of the animal, to perform some unknown function in the elaboration or purification of the blood.

Such a modification of a part of the embryonic kidney is by no means unique amongst vertebrates. In the last paper which he wrote for this Journal, the late Professor Balfour<sup>1</sup> showed that, at all events, in a considerable number of Teleostei the head kidney becomes in the adult transformed into a mass of tissue resembling a lymphatic; and he subsequently discovered the same modification in the head kidney of *Lepidosteus*.<sup>2</sup>

This being the case, the question arises whether there may not exist, in all vertebrate animals, similarly modified portions of the primitive kidney. I believe that such structures are, as a matter of fact, to be found in the suprarenal bodies.

Though this view of the nature of the suprarenals is by no means in accordance with that generally held, none of the facts at present known concerning either their adult relations or their mode of development seem to me to disprove it.

First, as to their relations in the adult.

In Elasmobranchs there are, as Balfour has shown,<sup>3</sup> two distinct sets of structures to which the name "suprarenal bodies" has been applied; first, a series of paired, apparently glandular bodies, arranged segmentally, and each connected with a sympathetic ganglion; these bodies, first accurately described by Leydig,<sup>4</sup> are attached on each side to the dorsal wall

<sup>1</sup> Balfour, on the Structure of the Organ known as the Head Kidney in Teleostei, this Jour., 1882.

<sup>2</sup> "On the Structure and Development of *Lepidosteus osseus*," by F. M. Balfour and W. N. Parker, 'Phil. Trans.,' 1882.

<sup>3</sup> 'Elasmobranch Fishes.'

<sup>4</sup> Rothen und Haie, Leipzig, 1852, 'Untersuchungen über Fische und Reptilien, 1853.



of the cardinal vein on each side, projecting into its lumen. They are best developed in the region of the mesonephros. In the region of the hind kidney, these bodies are replaced by a median, impaired structure, the lobuli composing it being closely connected on each side with the adjacent parts of the kidneys.

In Teleostei suprarenals are at all events frequently absent; or, as I would rather suggest, they are represented by the greatly metamorphosed head kidney described by Balfour.<sup>1</sup> In other cases, where suprarenals have been detected, they have always been attached to the surface of the kidney.<sup>2</sup>

In Amphibia, they are embedded in the substance of the kidney, either on its ventral surface (frog), or on its internal border (Triton); and they, like the kidneys, receive blood from the renal portal vein.<sup>3</sup>

In Reptiles<sup>4</sup> the adult structure of the suprarenals strongly supports the view that they are modified portions of the mesonephros. In the male lizard, for example, they lie suspended in the mesorchium, between the testis and the seminiferous Wolffian tubules, with which latter they are closely connected.

In snakes the relations are very similar, while there is a remarkably well developed "adrenal portal" circulation.

In Birds and Mammals the highly specialised suprarenals retain, as might be expected, fewer traces of their mode of origin than is the case in lower forms.

It is evident, from the above sketch of their relations, that the only case among lower Vertebrates, in which any well-marked separation between kidneys and suprarenals occurs, is among Elasmobranchs, where the anterior paired portion of the suprarenal system is very distinctly separated from the mesonephros. This fact, however, is probably due simply to the extreme degree of specialisation undergone by the meso-

<sup>1</sup> Loc. cit.

<sup>2</sup> Ecker, 'Der feinere Bau der Nebennieren,' 1846.

<sup>3</sup> Owen, 'Anatomy of Vertebrates,' vol. i, p. 543.

<sup>4</sup> Braun, "Bau u. Entwick. d. Nebennieren d. Reptilien," 'Arb. Zool. Inst.,' Würzb., 1872.

nephros in the male, where it forms the complicated network of the epididymis, while in the female it by no means retains its primitive characters.

In Amphibians and Reptiles the intimate connection of the two sets of organs, and the great similarity between their means of blood supply—each receiving a portion of venous blood from the trunk or hind limbs, which passes through the organ (kidney or suprarenal as the case may be), to go to the vena cava—are surely most easily explained by supposing both organs to be parts of a single primitive structure, which are undergoing specialisation in different directions.

The very general absence of suprarenals, as separate structures, in Teleosteans, together with the existence of a peculiarly modified head kidney, has already been mentioned as leading to the same conclusion. The connection between the suprarenals and more or fewer of the sympathetic ganglion, which exists in so many forms (Elasmobranchs, Reptiles, Birds, Mammals) can hardly be other than secondary.

The development of the bodies in question has been worked out in Elasmobranchs by Balfour,<sup>1</sup> in Reptiles by Braun,<sup>2</sup> and in Mammals by Mitsukuri<sup>3</sup> and Janosik.<sup>4</sup> In all these forms the first recognisable rudiment of a suprarenal is in the form of a compact mass of mesoblastic tissue, lying dorsal to the Wolffian body, between it and the aorta; and therefore just at the base of the ridge of the commencing generative epithelium. The cells composing this mass envelope a certain number of sympathetic ganglia; forming the cortical part of the adult suprarenal, while the cells of the ganglia form its central part. The question of the homologies of the cortical part of the suprarenals must, if it is to be settled by embryological evidence at all, be decided by observations on the mode of origin of the primitive cell-mass from which the cortical substance of the adult organ arises. On this point there is, however,

<sup>1</sup> 'Elasmobranch Fishes.'

<sup>2</sup> Loc. cit.

This Journal, Jan., 1882.

<sup>4</sup> 'Archiv für Mikroskopische Anatomie,' xxii Band, 1883.

very little evidence. Balfour and Mitsukuri give no definite account of the mode of origin of the cell mass, their observations beginning at a time when it is already formed. Braun considers that in Reptiles it commences by the formation of aggregations of cells round branches of the vena cava, while admitting<sup>1</sup> that "the rudiment . . . is often so close to the segmental tubules at their point of exit from the Malpighian capsules, that one is easily led to believe in the existence of a connection between the two." But the most striking observations on this point are those of Janosik, who finds that in Mammals there is, immediately in front of the Müllerian duct—that is, in the position of the head kidney—a number of solid cords of cells, connected at intervals with the peritoneal epithelium, and resembling exactly, so far as one can judge from the account given, a series of solid rudimentary segmental tubules. These strings of cells have no connection with the renal duct, but pass directly into the cortical substance of the suprarenals.<sup>2</sup>

Such fragmentary observations as I have hitherto been able to make lead me to hope that I may be able at no very distant date to show that, at all events in Reptiles and Mammals, the connection between the Wolffian body and the suprarenal is much more intimate than has generally been supposed. But should this hope prove unfounded, and should subsequent observations prove that the primitive mesoblastic rudiment arises simply as a mass of cells lying dorsal to the Wolffian body, this would by no means afford sufficient reason for asserting that the one structure had never been connected with the other, for we know that precisely the same kind of separation of two primitively continuous parts of the kidney has taken

<sup>1</sup> Loc. cit., p. 23.

<sup>2</sup> A short time before the appearance of Dr. Janosik's paper, Dr. Renson published in the 'Archiv für Mikroskopische Anatomie' (Bd. 22, p. 600), an account of some observations which tend to prove the presence, in earlier mammalian embryos, of functional segmental tubules in the position of the solid cords of Janosik. As neither of these authors figure the structures described, it is impossible to judge how far the one set may prove identical with the other.

place in the case of the metanephros, which, originally continuous with the hind end of the Wolffian body, now develops from a separate blastema which bears much the same relation to the hinder end of that structure as the suprarenal blastema does to its anterior end—both modes of origin being probably equally due to the delay which always takes place in the histological differentiation of an organ which is only functional comparatively late in life, and to the need of separating as soon as possible all such temporarily useless structures from the actively functional Wolffian body.

In the present state of our knowledge as to the function of the suprarenals, it may seem unjustifiable to assume that they have any essential connection with the blood system. At present, the only piece of direct evidence of their possessing any function at all is derived from the phenomena of Addison's disease, which seems, so far as I can learn, to be essentially due to alterations in the blood supply. The constant alterations in the behaviour of the red corpuscles, their refusal to form rouleaux, and the frequent difficulty in obtaining a good clot from the blood of patients suffering from this disease, are very suggestive.

An important indication of the probable need for some set of glandular structures in connection with the vascular system is found in the very general presence of such glands among the Invertebrates. It is not too much to say that in every group of Invertebrates in which the vascular system has been at all carefully investigated, glandular appendages to the vessels have been found, which can, from their anatomical relations, have no other function than that of elaborating some of the constituents of the blood. Thus, in Chætopods<sup>1</sup> there are very frequently present small cæcal diverticula of the great vascular trunks, which are coated with large, nucleated cells, loaded with granules; these cæca may simply lie loosely along the sides of the vessels, or may be collected into definite glandular masses lying on the floor of the body-cavity. In leeches,

<sup>1</sup> See Claparède, "Organisation des Annélides Sédentaires," and Cosmovici, "Les Annélides Polychètes," 'Arch. Zool. Exp.,' viii.



some glandular function may possibly be attributed to the large chains of "botryoidal connective tissue" in which many of the blood-vessels end.<sup>1</sup> In Echinoderms, the abundance of glandular cells in the cardiac plexus is probably a principal cause of the whole organ being regarded by many observers as an excretory apparatus. Among Molluscs, glandular structures, connected with the auricles, have long been known among Cephalopods, while the glands of the pericardium of Lamellibranchs, associated as they generally are with the auricles and afferent vessels, are probably of the same nature.<sup>2</sup> Among Arthropods, the "coxal glands," recently described by Professor Lankester,<sup>3</sup> may perhaps prove to be connected with the vascular system, though the small blood supply at present recognised is certainly against such a view.

An investigation of the functions of these various structures in Invertebrates can hardly fail to afford an important clue to the real nature of the Vertebrate suprarenals.

### EXPLANATION OF PLATE XV,

Illustrating Mr. W. F. R. Weldon's Paper "On the Head Kidney of *Bdellostoma*, with a Suggestion as to the Origin of the Suprarenal Bodies."

#### *Complete List of Reference Letters.*

*B.* Lining epithelium of a glomerulus. *b.-c.* Blood-corpuscles. *b.m.* Basement membrane. *c.* Blood capillaries. *cl.* Blood-clot in ventral duct of head kidney. *D. ep.* Epithelium of central duct. *gl.* Glomerulus at posterior extremity of head kidney. *g*<sup>1</sup>. First glomerulus of functional kidney. *g*<sup>2</sup>.

<sup>1</sup> Lankester, "On the Vasifactive and Connective Tissues of the Medicinal Leech," This Journal, vol. xx, 1880.

<sup>2</sup> See Grobben, "Morphologische Studien über die Harnund Geschlechts-apparat, &c., der Cephalopoden," 'Arb. Zool. Inst. Wien,' v Bd., 1883.

<sup>3</sup> "On the Skeletotrophic Tissues and Coxal Glands of *Limulus*, *Scorpio*, and *Mygale*," This Journal, Jan., 1884.

Granules attached to epithelium of central duct. *f.* Opening of head kidney tubules to pericardium. *Ly.* Lymphatic tissue at posterior end of central duct. *pc.* Pericardial epithelium. *S. D.* Segmental duct. *s. d.* Atrophied portion of segmental duct. *v. c.* Great vein. *x.* Strands of blood-vessels passing from glomerulus to lymphatic tissue of head kidney.

FIG. 1.—View of head kidney of *Bdellostoma*, from within the pericardium. The segmental duct is seen through the wall of the pericardium.  $\times 4$  diam.

FIG. 2.—Diagrammatic longitudinal section through head kidney of *Bdellostoma*.

FIG. 3.—Transverse section through the middle of the head kidney. The tubules and the central duct are drawn, but the capillaries surrounding the tubules are omitted. Zeiss, obj. A, oc. 2.

FIG. 4.—Epithelium of central duct, showing granules thrown into its lumen. Zeiss, Obj. F, oc. 2.

FIG. 5.—Clot from central duct. Obj. F, oc. 2.

FIG. 6.—Transverse section through the lymphatic tissue at the posterior end of the central duct, showing strands of blood-vessels passing into it from the adjacent glomerulus. Obj. D, oc. 2.

FIG. 7.—Portion of periphery of a section similar to that shown in Fig. 3, showing the characters of the head kidney tubules and the surrounding blood-vessels. Obj. D, oc. 2.

Figs. 3 to 7 drawn with the camera lucida.

## On some Points in the Minute Structure of the Pancreas.

By

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With Plate XVI, figs. 1, 2 and 3.

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IN the pancreas of the rabbit Kuhne and Lea have described accumulations of cells between the alveoli having a distinct blood supply. I have examined the pancreas in a large number of animals, such as dog, cat, guinea pig and ape, and I find that these agglomerations of cells are constant in all of them.

They lie amongst the alveoli in contact with them, but are not mixed indiscriminately with them. I have found in some parts a trace of fine connective tissue on their periphery, but nothing like a distinct capsule separating them from the surrounding alveoli.

Their peculiar arrangement is shown in Pl. XIV, fig. 1.

Each agglomeration consists of a number of polyhedral cells, each cell having a distinct nucleus; this nucleus is of irregular shape and stains very deeply.

The substance of each cell is filled with a very fine network of fibrils (Pl. XIV, fig. 2). Each mass consists of cells and capillary blood-vessels. I have not been able to find anything corresponding to centro-acinar cells amongst them, although they occur in all the surrounding alveoli. The blood supply is very marked, each mass of cells having a distinct capillary plexus, as has already been described by Kuhne and

Lea. This blood supply is very much greater than that of the surrounding alveoli, and appears to be confined to these agglomerations of cells. The capillaries are larger than those of the alveoli, and in an injected specimen mark out these masses of cells very distinctly.

Another feature is their behaviour to staining reagents. With ordinary logwood solution, the only difference is that the nuclei color very deeply, much more so than the nuclei of the alveolar cells.

By the use of other staining agents these cell masses can be stained a different colour to the alveoli surrounding them.

The best stains for this purpose I have found to be vesuvin and sulphindigotate of soda.

The pancreas should be hardened in chromic acid to get the best results, as after spirit hardening the sections are so delicate they will not stand the different processes. The sections are first placed in a watery solution of vesuvin for ten minutes; a 10-per cent. solution made with distilled water and diluted one half is the best.

The sections must be well washed in distilled water to get rid of the superfluous colour. They are then placed in a 5-per cent. solution of sulphindigotate of soda until they become a deep blue colour.

They are then well washed in water and afterwards in spirit, and mounted in Canada balsam.

It requires some practice to get the right shades of colour, but when this is done the difference between the two tissues is most marked.

This double stain is also well adapted for other organs.

By this method of staining it can be seen that different portions of the pancreas are in different states of activity. A few alveoli will be seen distinctly mapped out from the rest by the way in which their cells have stained; they show the two zones very well.

With regard to the significance of these agglomerations of cells, it seems scarcely probable, taking into consideration their distribution amongst so many animals and their distinct blood



supply, that they can be merely the remains of embryonic tissue.

Looking at the diverse functions of the pancreas may they not take some share therein?

I have not yet had an opportunity of injecting the human pancreas.

Another peculiarity in the pancreas, which so far as I know has not been recorded, occurs in the duct.

After the intermediary part the duct becomes lined with columnar cells, at first short, then longer, a fibrous connective-tissue sheath is gradually formed round the duct. This becomes thicker as the duct approaches the surface.

At some distance from the surface the duct is very large and of an oval shape. It is lined by a single layer of columnar cells; these rest on a varying amount of fibrous connective tissue. Between this lining epithelium and the fibrous connective-tissue sheath, there is a complete circle of mucous glands; they consist of distinct tubes lined by large cells having a well-defined network. The different alveoli are separated by fibrous trabeculæ continuous with the outer sheath (see Pl. XIV, fig. 3).

The ducts having these mucous glands vary very much in size, one section will often show two ducts one three times as large as the other. The drawing was made from one of the largest.

These mucous glands have a very large blood supply, there being a dense plexus of capillaries between them and the lining epithelium and also on the outside under the fibrous sheath.

They are very well seen in the pancreas of the guinea-pig.

In a pancreas of the cat I found a small accessory spleen, which had grown round some of the alveoli and isolated them from the rest of the organ; a single minute duct from the pancreas lined by columnar cells passed through it.

In another part of the same pancreas there was a small mass of splenic tissue surrounded by a fibrous capsule, and in its substance were several branched tubes from the pancreas.

The whole of this lay amongst the pancreatic alveoli; the first described was attached to the side of the pancreas.

**On some Structures found in the Connective  
Tissue between the Renal Artery and Vein  
in the Human Subject.**

By

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School of the Westminster Hospital.

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With Plate XVI, figs. 4 and 5.

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IN dissecting out the renal artery and vein in a human subject I found some small bodies which I at first thought were lymphatic glands. On hardening and making sections of them, I found that they consisted of different structures. Some of them were small lymphatic glands, differing in no way from similar glands in other parts; the largest was about the size of a pea, while others were much smaller.

One round body, about the size of a hemp-seed, turned out to be a ganglion. It was nearly circular in shape, and had a well-marked fibrous capsule, outside this were several nerve-trunks consisting of medullated nerve-fibres; the nerve-trunks varied very much in size. Inside the capsule were a large number of ganglion cells, each cell lying in a single capsule formed of a nucleated endothelial membrane.

Amongst the ganglion cells were a number of nerve-bundles consisting of medullated and non-medullated fibres. The whole was well supplied with blood-vessels.

The next structure consisted of an oval body measuring a little over a quarter of an inch in length by an eighth of an inch in breadth. It had a fibrous capsule which varied very much in

thickness in different parts of the circumference. This capsule sent in fibrous trabeculæ dividing the cortical portion into alveoli.

But these trabeculæ varied very much in their size and arrangement, being in some places very much thicker than the fibrous capsule itself, while in other places they were mere threads of fibrous connective tissue. They also varied in their distance from one another; in some places being close together, in others wide apart.

In the alveoli were masses of round cells resembling large lymph corpuscles. These masses varied very much in size. In many of them were fibrous trabeculæ, seen in transverse section, passing through the substance of the mass.

From this description of the cortical portion it will be seen that there is some resemblance to an ordinary lymphatic gland, but in the medullary portion the resemblance ceases.

The fibrous trabeculæ in the medullary portion, or that portion of this structure which corresponds to the medullary portion in an ordinary lymphatic gland, are very much thicker than in the periphery, while the masses of cells have the same irregular arrangement as in the periphery.

This will be seen from the drawing made with a low power (Pl. XIV, fig. 4).

Throughout all parts of this structure the cell masses are separated from the fibrous trabeculæ by a well-defined space similar to the lymph space in a lymphatic gland, but no trace of the reticulum occupying this space in the ordinary lymphatic gland can be seen. A few fibres pass across the space from the trabeculæ, but they are very few, and appear to be merely isolated fibres of connective tissue.

Throughout the whole of the fibrous trabeculæ are a very large number of blood-vessels, varying from an artery with a well-defined muscular coat to large capillary vessels.

The great peculiarity of this structure consists in these blood-vessels opening directly into the spaces between the cell masses and fibrous trabeculæ. (See Pl. XIV, fig. 5.)

In all parts these spaces are seen to contain red blood-

corpuscles, and blood seems to come from the vessels in the trabeculæ which open into these spaces, and to circulate through them. It will be seen, then, that although in the arrangement of the component parts of this structure there is a certain resemblance to a lymphatic gland, there is this great difference—in the one a current of lymph passes through the spaces between the cell masses and trabeculæ, while in the other there is a current of blood.

Some of the spaces, especially in the periphery, are filled with red blood-cells; amongst these are a number of much larger cells, each containing a well-marked nucleus. This nucleus is as large as that of the cells forming the cell masses, but does not stain as deeply.

In the cell masses the cells are closely aggregated together, and only their nuclei can be made out.

The large cells are much more numerous in the spaces near the periphery than in the centre.

In one part of the capsule I noticed a capillary vessel passing through a space lined by endothelium. The capillary was empty, but the space was filled with red blood-corpuscles.

The above structures were found in the body of an adult male under 30, who died from the effects of an accident.

The next case I examined was that of a man aged 58, who died from phthisis.

I found the same structures as in the previous case. The ganglion, however, was much larger, and each ganglion cell contained a large number of yellow pigment granules.

In this case I found three of the glands described before. They varied in size, two being much smaller than the one found in the first case, while the third was much larger, about double the size. They were, however, identical in structure with the one already described, and all the spaces between the cell masses and trabeculæ were filled with red blood-corpuscles, and amongst them large nucleated cells.

In addition to these I found in this case an oblong body measuring half an inch in length. In structure it exactly resembled a suprarenal capsule.



Many of the sections showed large nerve-trunks passing through the parenchyma. I did not find a similar gland in either of the other cases.

The third case was that of a male aged 19, who died of phthisis.

In this I also found a small ganglion very similar to that of the first case. The gland resembling a lymphatic was also present, and showed a similar structure to that described in the first case.

There was only one ordinary lymphatic gland in this case, but it was much larger than any of the others.

In neither of these three cases was there any disease of the kidneys.

From the above it will be seen that I have found this peculiar gland, resembling a lymphatic gland somewhat, but having blood circulating in it instead of lymph in three cases, and these were consecutive; it would, therefore, seem to be a permanent structure.

## EXPLANATION OF PLATE XVI.

Illustrating Dr. Gibbes' papers "On Some Points in the Minute Structure of the Pancreas," and "On Some Structures found between the Renal Artery and Vein."

FIG. 1.— $\times 52$ . Alveoli of the pancreas of the guinea-pig. *a*. Agglomerations of cells with large and distinct blood supply. *b*. Normal pancreatic tissue. *c*. Coagulated effusion into interalveolar spaces.

FIG. 2.— $\times 680$ . Cells from agglomeration (*a*, fig. 1) isolated.

FIG. 3.— $\times 120$ . Circle of mucous glands surrounding a large duct of the pancreas of the guinea-pig. *a*. Normal pancreatic tissue. *b*. Fibrous connective tissue surrounding duct. *c*. Chain of mucous glands. *d*. Columnar epithelium lining duct.

FIG. 4.— $\times 56$ . Glandular body occurring between the renal artery and vein in man. The drawing shows the general arrangement of fibrous trabeculae, cell masses, and the channels between them. *a*. Fibrous capsule continuous with trabeculae throughout the gland. *b*. Masses of cells resembling lymph-corpuscles. *c*. Spaces between cell masses and trabeculae in which blood is circulating.

FIG. 5.— $\times 520$ . Portion of the preceding more highly magnified. *a*. Fibrous trabeculae. *b*. Cell masses. *c*. Space between these which contains blood. *d*. Blood capillary opening into the space between cell masses and trabeculae.

## Histological Notes.

By

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### I.—CILIATED EPITHELIUM IN THE KIDNEY.

IN 1880 Dr. Klein found ciliated epithelium in the kidney of a mouse. He has published an account of this in the April number of this Journal for 1881.

He describes the cilia as occurring in the convoluted tubes near the Malpighian corpuscles.

At Dr. Klein's suggestion I carried out this inquiry in the kidneys of different animals. I found them in the kidney of the white rat, brown rat, guinea-pig, and dog.

It is necessary to harden the kidney in alcohol to bring out the cilia clearly; if chromic acid or Müller's fluid is used, they cannot be made out so well.

To show them well the sections must be very thin; they may be cut with the Williams' freezing microtome, and care must be taken in transferring them from the different reagents.

The cilia appear to be in that portion of the convoluted tube near the Malpighian corpuscles, but I have also found them in transverse sections of tubes which may have been further away.

They are short and very fine, set densely on the free edge of the cell.

A magnifying power of 500 diameters shows them very well.

Mr. J. W. Groves, of King's College, has also observed cilia in the kidney of the dog.

I next examined a number of kidneys from the human foetus about full time. In every case where the kidney was fresh and had been hardened in alcohol, I found the cilia in the convoluted tubes. They seemed to be much more widely distributed than in the adult animals I had previously examined. They were to be seen in many more tubes in one field of the microscope, and they were present in a large number of transverse sections of convoluted tubes. It appeared, in fact, as if they were present in all the convoluted tubes, proximal and distal. They are very minute, and there are a large number on each cell; they require great care in the preparation to show them well, and the sections must be very thin. I found logwood to be the best stain to bring them out.

The kidney must be perfectly fresh.

I examined a large number of adult human kidneys without finding any cilia in the convoluted tubes. I attributed this to the difficulty of obtaining them in a perfectly fresh state. I found traces in some, but it was not until last year (1882) that I succeeded in getting them out satisfactorily.

A case of progressive pernicious anæmia was sent me for examination, and on making specimens of the kidney after hardening, I found that numbers of the convoluted tubes contained cilia.

They resembled those in the foetal human kidney, but were not so widely distributed. They are shorter than those found in the kidney of the mouse and rat, and they are very much finer and more numerous than the cilia on the cells of the human trachea.

In the adult kidney they stained deeply at the base, giving the appearance of a dark line at the free edge of the cell.

They can be easily seen with a  $\frac{1}{8}$ th objective, but the binocular microscope, with either a  $\frac{1}{6}$ th or  $\frac{1}{8}$ th, brings them out very plainly.

Professor Tuttle, of Ohio, has observed cilia in human kidneys from cases of smallpox, also in kittens.



## II.—STRIPED MUSCULAR TISSUE ATTACHED TO HAIR FOLLICLES.

While making an examination into the structure of the tactile hair follicles, I found that they are moved by muscles composed of striped muscle tissue, and that they differ in this from the ordinary hair follicle.

These striped muscle fibres are very long and slender; they vary in number; in some parts there are only two or three, in others seven or eight. They have no special fibrous tissue surrounding them, but run side by side in the connective tissue round the follicle.

They are inserted into the follicle near the base, on either side. Their insertion appears to extend for some little distance round the base, and in the central portion of the insertion the most fibres are found. They run to the surface close by the side of the follicle at its wide part, but where it narrows they suddenly diverge, and, proceeding obliquely, terminate in the fibrous tissue.

In many cases they can be traced to the fibrous tissue lying under the superficial epithelium.

On the Laticiferous Tissue of *Manihot*  
*Glaziovii* (the Cearà Rubber).

By

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(From the Jodrell Laboratory, Kew.)

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With Plate XVII.

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IN the course of some work on the development of laticiferous tissue in the secondary cortex I was led to examine the Cearà Rubber (*Manihot Glaziovii*), a plant to which my attention was first directed by Mr. Thiselton Dyer. The investigation of this plant has led to some very unexpected results, which it is the object of the present paper to communicate.

It has hitherto been assumed, expressly or tacitly, that in those families which possess any kind of laticiferous tissue the articulated and the non-articulated forms mutually exclude one another. Thus, De Bary<sup>1</sup> states that "each of these categories is peculiar to definite families," while the same conclusion follows from Sachs' treatment of the subject in his 'Textbook'<sup>2</sup> and 'Lectures.'<sup>3</sup> Although it is well known that, in the same family, genera with and without laticiferous tubes may occur, as in the Papaveraceæ and Aroideæ,<sup>4</sup> yet, where present at all, this tissue has hitherto been found to be of uni-

<sup>1</sup> 'Vergleichende Anatomie,' p. 195.

<sup>2</sup> 2nd Eng. ed., pp. 85, 86.

<sup>3</sup> 'Vorlesungen,' pp. 206, 207.

<sup>4</sup> De Bary, l. c., pp. 450, 451.

form character throughout each natural order. My observations on the genus *Manihot* show that the Euphorbiaceæ form an exception to this rule. I have been able to prove that in *Manihot* the laticiferous tissue consists of vessels, while in the members of the order previously investigated it only occurs in the form of the most typical laticiferous cells. The subsequent note on *Hevea* will show that the former structure is not confined to one genus, but also reappears elsewhere in the order. The laticiferous tissue of the Euphorbiaceæ, except in *Euphorbia* itself, is so little known that further researches may not improbably disclose other important variations.

It will be desirable in the first instance to give a general account of the distribution of the laticiferous tissue in the different parts of the plant. All the following statements refer to *M. Glaziovii* (the species which I investigated most completely), except where otherwise stated.

In the young stem, 3 mm. or less in diameter, at the commencement of secondary thickening, the laticiferous tubes form two series. One, which is much the less developed of the two, is hypodermal,<sup>1</sup> occurring in or about the third layer from the epidermis. The other series lies in the ring of bundles, from four to six layers of cells within the bundle-sheath, which is here "compound," consisting partly of sclerenchymatous fibres, and partly of cells which contain klinorrhombic and clustered crystals of calcium oxalate, and which subsequently undergo sclerosis. The inner system of laticiferous tubes is continuous all round the stem, occurring in the interfascicular tissue as well as in the phloëm of the five primary bundles of the internode. As secondary thickening goes on in the stem, additional concentric series of laticiferous tubes successively appear in the secondary cortex.

In the petiole, besides the laticiferous tissue in the phloëm of the bundles, there is also a hypodermal system traversing the thick layer of collenchyma which lies between the epi-

<sup>1</sup> This is mentioned by Trimen. See 'Report on Progress, &c., of the Royal Gardens, Kew,' for 1881, p. 14.

dermis and the bundle-sheath. Here the tubes themselves have in many cases thick, collenchymatous walls.

In the middle nerves of the three segments of the leaf, a zone of laticiferous tissue extends all round the vascular bundles, occurring inside the starch-sheath which here surrounds them. Here, as in the petiole, a hypodermal system is also present, which traverses the collenchyma of the upper and lower surface of the nerve. In the smaller veins of the leaf the tubes accompany the vascular bundles on their lower side.

In the young tetrarch or pentarch root the laticiferous tissue is confined to the outer part of the four or five phloëm groups. It does not occur in the hypodermal position.

In the thickened tuberous root the laticiferous tubes occur in the secondary cortex, forming, together with the sieve-tubes, a coarse network, enclosing the large medullary rays.

I will now return to the secondarily thickened stem. On examining transverse sections, the laticiferous tubes of the phloëm are seen to be very numerous. They appear to be connected one with another in the tangential direction by transverse branches, especially in the outer part of the phloëm. The observation of a tangential section through this region at once shows that this is actually the case, and thus discloses the first important difference between the laticiferous tissue of *Manihot* and that of *Euphorbia*. The occurrence of anastomosis in the latter has always been doubtful. Schmalhausen,<sup>1</sup> indeed, believed that it probably occurs in the nodes, young leaves, apex of the root, &c., but De Bary<sup>2</sup> considers this questionable in every case, and only admits its possibility as regards the nodes. The most recent investigations are those of Schullerus,<sup>3</sup> who comes to the conclusion that, in *E. Lathyris* at least, anastomosis does not take place at all, either in the nodes or elsewhere. Here, in the cortex of *Manihot*, anastomoses are extraordinarily frequent, and often very regularly arranged.

<sup>1</sup> 'Beitr. z. Kenntniss d. Milchsaff-behälter d. Pflanzen,' St. Petersburg, 1877.

<sup>2</sup> L. c., pp. 199, 205.

<sup>3</sup> 'Verhandl. d. Bot. Vereins d. Prov. Brandenburg,' 1882, 2, p. 92.



By means of these the main trunks of the laticiferous tubes are everywhere connected one with another in the transverse direction. The connection takes place in different ways in the primary and in the secondary phloëm. In the former the laticiferous tubes sometimes occur singly, separated by single layers of parenchymatous cells, as seen in tangential section. This is especially the case in the outer regions of the phloëm (see fig. 2). In other cases they form bands, each of which consists of two, three, four, or more tubes, and these bands are connected to form a network with long meshes. The individual tubes forming one of these bands are connected by very numerous perforations of their lateral walls. These may be so numerous that the greater part of the boundary wall disappears, only narrow transverse bands of cellulose being left between the perforations. In other parts of their course tracts of considerable length may be found without any perforations. The single tubes separated by parenchyma, or the constituent tubes of neighbouring bands, are connected in two ways. Sometimes simple transverse branches extend between the parenchymatous cells from one tube to the other (fig. 2). In other cases complicated anastomosing knots are present, in which several transverse branches are in contact, and united among themselves by perforations of their horizontal walls (fig. 4).

The laticiferous tubes of the secondary phloëm also occur in bands, which are connected to form a network, the meshes of which are occupied by the medullary rays. The tubes of each group are connected among themselves by numerous lateral perforations, as in the primary phloëm, and when the bands meet at the top and bottom of a ray their tubes also anastomose, so that open communication, in the tangential direction, exists throughout the entire network. The medullary rays of the secondary phloëm are not traversed by branches of the laticiferous tubes, so that the frequent transverse connections of the primary phloëm are absent here. Anastomoses in the radial direction were not observed, either in the primary or secondary phloëm.

The observation of the facts just stated is sufficient to show that the laticiferous tissue of *Manihot* is essentially different from that of *Euphorbia*, which has hitherto been regarded as in this respect typical of the order. The whole appearance of the tubes is quite different from that of laticiferous cells. There are no long, blindly ending branches, and the general character of the tissue is closely similar to that of the laticiferous vessels in the *Cichoriaceæ*. That cell-fusion takes place throughout the laticiferous tissue of *Manihot* is obvious even on inspecting it in its mature state, from the innumerable perforations which are present in the lateral walls. It remained, however, to be proved whether the individual tubes themselves are derived each from a single cell, or whether they are the product of the fusion of longitudinal series of cells.

The investigation of radial sections extending to the cambium, from a stem in which secondary thickening is in active progress, leaves no doubt as to the true character of these tubes. In the innermost zone of laticiferous tissue, in the immediate neighbourhood of the cambial layer, the transverse walls in the vessels are easily seen. In some the walls are still completely preserved, in others there are small perforations, through which the contents are already continuous, a decided constriction marking the point where they traverse the partially absorbed wall. In somewhat older stages only a narrow rim of cellulose remains, projecting into the lumen of the vessel. Tangential sections which exactly pass through the youngest region of the secondary cortex are still better adapted to show the mode of development of the tissue in question, as every stage of perforation of the transverse and longitudinal walls, leading to the formation of the continuous network of tubes above described, can be observed (fig. 3). The cells of which the laticiferous vessels are built up, are of about the same proportions as neighbouring cells of the phloëm parenchyma.<sup>1</sup>

<sup>1</sup> Methylene blue, which stains the cellulose walls only, is very useful in demonstrating the various stages of perforation. I have to thank my friend

In tangential sections of younger portions of the stem, in which the primary phloëm is still in course of development, the origin of the anastomosing knots mentioned above may be traced. One or more of the parenchymatous cells lying between two bands of laticiferous vessels becomes divided up by transverse walls into a number of narrow cells. The terminal walls of these cells, where they abut on the main trunks of the vessels, become wholly or partially absorbed, and the same is the case with large portions of their horizontal walls. A complicated system of communicating passages is thus produced (fig. 4).

Very complicated anastomoses also take place at the points of junction of the meshes formed by the laticiferous vessels. At these places a large number of vessels are in contact, and they may all have the longitudinal walls separating them absorbed, at numerous points, only quite narrow bands of cellulose being left between the perforations.

As regards the period at which perforation of the walls takes place, two facts may be mentioned which illustrate the close similarity between the laticiferous tissue in *Manihot* and that in the *Cichoriaceæ*. The first point is that cross-walls may be found still completely preserved, where large perforations in the lateral walls already exist (cf. fig. 3). Among the *Cichoriaceæ* it was noticed by Schmalhausen<sup>1</sup> in the case of *Tragopogon*, that the lateral walls are perforated before the transverse ones, and this observation I was able to confirm in most, if not in all cases.<sup>2</sup> Secondly, in *Manihot*, as in the *Cichoriaceæ*, latex is present before the cross-walls are absorbed.<sup>3</sup>

The lateral walls of the laticiferous vessels are often much undulated, the protrusions and depressions fitting into one another where two vessels run side by side. It follows that after perforation has taken place, the remaining portions of the

Mr. W. Gardiner for calling my attention to this reagent, and for other practical suggestions.

<sup>1</sup> L. c.

<sup>2</sup> See vol. xxi of this Journal (1882), p. 147.

<sup>3</sup> Ibid., p. 152.

walls lie in various directions, a fact which gives an additional appearance of complexity to parts of the mature tissue. Where a vessel is in contact with parenchyma, short pointed protrusions are very often wedged in between the cells of the latter. But these protrusions never grow out into branches of any considerable length, like those so often described in the case of the Cichoriaceæ, &c. This fact is of some interest, as the occurrence of these protruding branches is the only certain point in which the laticiferous vessels at all approach the laticiferous cells, and it is remarkable that here, where indications of a transition might perhaps have been expected, they should be absent.

The hypodermal system of laticiferous tubes agrees in the main points of structure with that in the outermost regions of the phloëm. The main trunks of the vessels, which are here on the whole further apart, are connected into a network by branches running more or less accurately in the transverse direction (fig. 1). The development of the tissue in this region is altogether more scanty than in the phloëm, as stated above. I have not especially investigated the mode of origin of this portion of the tissue, but the reticulate arrangement leaves little doubt that it is the same as in that of the phloëm.

Considering the great development of the laticiferous tissue in *Manihot*, a corresponding reduction of the sieve-tubes would be expected. This at least is the conclusion to which one would be led by the prevailing views as to the relation between the two tissues, as expressed, for example, by De Bary, especially at p. 541 of his '*Vergleichende Anatomie*.' As a matter of fact, however, the reverse is the case in *Manihot*, as the sieve-tubes are remarkably well-developed, both in the primary and secondary bast. In the former they have the horizontal or slightly inclined walls usual in this region, each with one sieve-plate. They usually attain a diameter of 0.026 mm. In the secondary phloëm their terminal walls are inclined to the radial plane at an acute angle, and each wall is occupied by a single series of numerous sieve-plates. The plates show the connecting strands of protoplasm, callous deposits, &c., remarkably



well. I have measured secondary sieve-tubes with a diameter of .031, .035, and .04 mm. respectively. The former dimensions are more usual. The sieve-tubes are accompanied by narrow companion-cells with the typical characteristics. In the primary sieve-tubes of the younger portion of the stem I have occasionally found nuclei. The members of the sieve-tubes seem to be multinucleate, as in one case at least I have counted three nuclei in a single element.

As regards the relative positions of the laticiferous tubes and the sieve-tubes, no very strict regularity prevails. Generally speaking, they form alternating concentric rings. As many as three sieve-tubes may occur in contact in the same radial row. Laticiferous tubes and sieve-tubes are very commonly separated by a single cell of the phloëm-parenchyma. In a few cases I have found actual contact between the two.

As regards the occurrence of nuclei in the laticiferous vessels, I have not found *Manihot Glaziovii* to be an especially favourable object for investigation. The presence of nuclei in the transverse connecting branches is easy to determine, but I have not often succeeded in demonstrating their presence in the main longitudinal trunks. I have found chloroform a useful reagent in this investigation, as it dissolves out the caoutchouc forming the bulk of the latex. The sections are transferred from absolute alcohol to a test-tube containing chloroform, in which they are shaken up and then left for about half an hour. They are then washed in absolute alcohol. The latex is found to have practically disappeared. In some cases I have been able to find traces of protoplasm and small nuclei in such preparations on staining with hæmatoxylin, but in others the tubes appear to be quite empty, so that the constant presence of a living protoplasmic body in the mature vessels would seem to be questionable. I should be sorry, however, to lay any weight on purely negative results, and this question must be reserved for renewed investigation.

I have made a general investigation of the laticiferous tissue in *M. utilisissima* and *M. Aipi*, and have found it to agree

<sup>1</sup> Cf. Russow, 'Dorpater Naturforscher Gesellschaft,' 1882, p. 282.

very closely with that of *M. Glaziovii*. The above statements may, therefore, probably be extended to the genus as a whole without any great risk of error.<sup>1</sup>

The results of my observations may be summed up as follows :

1. In *Manihot* the laticiferous tubes are not cells, as in the members of the order *Euphorbiaceæ* hitherto investigated, but vessels, agreeing in most points of distribution, structure, and development, with those of the *Cichoriaceæ*.

2. The high development of the laticiferous system is not inconsistent with the presence of large, numerous, and well-developed sieve-tubes. Hence, the prevalent views as to the mutual substitution of these two classes of organs are, to say the least, of limited application.<sup>2</sup>

In conclusion, I may add one or two general observations on the first of the two results just stated. The proof that in one and the same natural order both articulated and non-articulated laticiferous tubes may occur, shows that the structure of the tissue in question is of less importance as an "anatomical character"<sup>3</sup> than has hitherto been supposed. The presence of laticiferous tissue is evidently a character which has made its appearance repeatedly at distinct points in the phylogenetic development of the *Phanerogams*. This follows from the occurrence of the articulated tubes in groups so remote from one another as the *Cichoriaceæ*, *Papaveraceæ*, and *Aroidæ*, and of the non-articulated tubes in, for example, the *Euphorbiaceæ* and *Asclepiadeæ*. The results of the present investigation seem to me to render it probable that even within a comparatively narrow circle of relationship the development of laticiferous tissue has had more than one starting-point. In the order *Euphorbiaceæ* I should be disposed to assume a

<sup>1</sup> In Möller's short description of the cortex of *M. carthagenensis* there is no mention of laticiferous tissue ('*Anat. d. Baumrinden*,' p. 299).

<sup>2</sup> I may mention *Periploca* as a further well-known example of the simultaneous presence of an extensive laticiferous system, and well-developed sieve-tubes.

<sup>3</sup> Cf. '*De Bary*,' l. c., p. 27.

distinct origin for the laticiferous cells, such as those of *Euphorbia*, *Jatropha* or *Hura*, and for the laticiferous vessels such as I found to occur in *Manihot*. That the one form has been derived from the modification of the other is improbable. The essential distinction between the two seems to me to consist, not so much in the fact that the laticiferous vessels are originally septate, while the cells are not so, as in other phenomena of their development. The points on which I am inclined to lay most weight are the following. The vessels, except where they form protruding branches (which has not been found to take place in *Manihot*), occupy from their first origin the same position relatively to the surrounding tissues as they maintain in the mature condition. The laticiferous cells, on the other hand, are constantly, throughout their whole period of development, forcing their way into new regions, by penetrating between the cells of the tissues which surround them. This power of independent growth is universally characteristic of the laticiferous cells, and on it their entire development depends. In the case of the vessels it only appears as an inconstant and secondary phenomenon, and, as shown by cases like *Chelidonium* and *Manihot*, the complete formation of the laticiferous system may take place without it.

A second point of distinction lies in the early differentiation of the laticiferous system from all other tissues, which occurs in the case of the cells only. As shown by Schmalhausen (l. c.), small number of cells, already formed in the embryo, give rise to the entire laticiferous tissue of the plant (if that in the secondary cortex be left out of consideration). This is not the case with the vessels. It is true that here also we find the rudiments of laticiferous vessels present in the embryo before germination, but from these rudiments only an insignificant fraction of the laticiferous tissue of the mature plant is derived. Laticiferous elements are here constantly being formed anew in the meristem of the various growing points as long as growth goes on. In other words, the differentiation of the laticiferous cells is simultaneous, while that of the vessels is successive.

To these points of difference may probably be added the absence of anastomoses in the case of the cells.

Taking all these facts into consideration it appears necessary, at least until transitional forms have been shown to occur, to regard the two categories of laticiferous tubes as distinct forms of tissue, which are only physiologically related to one another. And if this be so, it will follow, as suggested above, that the formation of laticiferous tissue is a phenomenon which has appeared independently, at least twice, within the circle of relationship now represented by the natural order Euphorbiaceæ.<sup>1</sup>

## EXPLANATION OF PLATE XVII.

Illustrating Mr. D. H. Scott's Memoir on the "Laticiferous Tissue of *Manihot Glaziovii* (the Cearà Rubber.)"

FIG. 1.—Hypodermal laticiferous tissue from young stem. Tangential section.  $\times 360$ . *l*. Laticiferous vessels. Many of the parenchymatous cells contain clustered crystals of calcium oxalate, indicated at *c*.

FIG. 2.—Laticiferous tissue from the primary phloëm of a stem about 1 cm. thick. Tangential section.  $\times 270$ . *l*. Laticiferous vessels. *t*. Their transverse connecting branches. *p*. Parenchyma.

FIG. 3.—Portions of young laticiferous vessels from an inner layer of the secondary phloëm. Tangential section.  $\times 460$ . At *t* and *t'* the transverse walls are preserved. At *t''* a rim of cellulose marks the position of an absorbed transverse wall. Several perforations in the lateral wall are visible. In the vessel to the right the transverse walls have almost disappeared. *m*. Medullary ray.

FIG. 4.—Tangential section through the primary phloëm of a young stem, showing an "anastomosing knot" between the laticiferous vessels.  $\times 360$ . The continuation of the vessel ending at *a* lay in a different plane from that of the section. *m* parenchyma resembling medullary rays. Its cells contain starch, which is indicated in two of the cells.

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As an example of a phénomène which has probably appeared repeatedly within a group of moderate extent, that of Heterospory may be cited. Cf. Goebel, 'Grundzüge der Systematik,' p. 313, &c.



## Note on the Laticiferous Tissue of *Hevea spruceana*.

By

**D. H. Scott, M.A., Ph.D.**

(From the Jodrell Laboratory, Kew.)

THE genus *Hevea* belongs to the sub-tribe *Jatropeæ* (Tribe *Crotonæ*, N. O. *Euphorbiaceæ*), according to the classification adopted in Bentham and Hooker's *Genera Plantarum*. I was not able to obtain material of the most important commercial species, *H. brasiliensis*, the Para rubber, as none could be spared just now from the Kew collection, but it is not probable that important differences exist between this species and *H. spruceana*, as regards the subject before us.

In the stem of the latter plant the general distribution of the laticiferous tissue is similar to that in *Manihot*. There is a somewhat scanty system of laticiferous tubes in the outer cortex, while within the bundle-sheath, which here also is "compound," consisting of sclerenchymatous fibres and sclerotic cells, the tubes are very abundant. They occur with equal frequency in the primary and in the secondary phloëm; the latter is here formed in considerable quantity.

In the petiole also laticiferous tissue is present both in the phloëm of the bundles and in the external parenchyma.

In the polyarch (adventitious) roots, the laticiferous tubes are confined to the phloëm groups, and have not been found in the outer cortex.

Tangential sections through any of the regions in which the laticiferous tubes occur, show that the latter form a complex anastomosing network.

In the outer (primary) portion of the phloëm of the stem, the tubes occur either singly, as is most frequent, or two side by side. Where the latter is the case, the intermediate longitudinal wall shows frequent perforations. The main trunks are connected into a very regular reticulate system by numerous transverse branches, passing between the parenchymatous cells.

In the secondary cortex the laticiferous tubes occur two or more together, forming bands which enclose the medullary rays. At the upper and lower ends of the latter the bands join. The tubes are connected among themselves by extremely numerous perforations in the lateral walls. These perforations are more numerous even than in *Manihot*, and it is quite usual for only short fragments of the wall to be left between them. These fragments appear swollen. Where several tubes meet at the points of junction of the meshes of the network, they are all in the freest possible communication one with another by means of these perforations.

The laticiferous tissue of the outer cortex is very sparse compared to that within the bundle-sheath, and hence anastomoses are comparatively infrequent. But they occur here as well as in the phloëm, and where two or three tubes run side by side, as sometimes happen, they anastomose in a complex manner, the intermediate walls showing numerous perforations of various dimensions.

On observing the young laticiferous tissue in the neighbourhood of the cambial layer, I found that the cells from which it is derived abut on one another with very oblique walls (inclined to the radial plane). These walls do not become wholly absorbed, but break down at numerous points. It is not improbable that transverse septa are also present in the earliest stages of development.

Although my observations on the development are thus by no means complete, yet those which I have already made, taken in connection with the whole structure of the mature tissue, leave no doubt at all on my mind that we have here once more to do with laticiferous vessels, and not with latici-

ferous cells. This conclusion is of special interest, as in the closely allied *Jatropha* the laticiferous tubes are most characteristic, typical cells, so far at least as can be decided without investigation of the embryonic development.

I hope to publish more detailed observations on *Hevea*, with figures, on a future occasion.

The Early Stages in the Development of  
*Balanoglossus* (sp. incert.).

By

**William Bateson, B.A.,**  
Scholar of St. John's College, Cambridge.

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With Plates XVIII, XIX, XX and XXI.

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THROUGH the great kindness of Dr. W. K. Brooks and the Council of Johns Hopkins University, I was permitted to avail myself during the summer of 1883 of the facilities offered by the Chesapeake Zoological Laboratory, which was then situated at Hampton, Virginia. My best thanks are due to Dr. Brooks for the most valuable and manifold assistance which he gave me, both in my work and otherwise, during my whole stay at the Chesapeake Station.

While resident at Hampton I had an opportunity of obtaining a small species of *Balanoglossus*, which was to be found there in great numbers, buried in the sand at about half tide.

The characters of the adult animal agree very closely with the description given by Agassiz of the species named by him *B. Kowalevskii*; but as the species which I examined developed in a manner totally different from that which Agassiz has described for *B. Kowalevskii*, I am compelled to suppose that the two animals are not identical.

As will afterwards be shown, the points in which the anatomy of this animal differs from that described by Agassiz are of such a fundamental character that it is impossible to regard them as



specific variations. Most of these points are in accordance with the account given by Spengel for *B. minutus* and *B. clavigerus*.

The occurrence of another species of *Balanoglossus* (*B. aurantiacus*) on the North American coast has been mentioned by Professor Leidy in a paper—which I have not been able to see; this species may possibly be identical with the specimens I have examined. On the whole, therefore, I have thought it better to leave the name of the species an open question.

In this paper I propose to deal only with the history of the early stages in the development of the animal, up to the formation of the layers and the commencement of the nervous system, and to describe very briefly the most important external changes which occur in later larval life so far as I have been able to observe them. I hope shortly, however, to give an account of the subsequent development of the organs, and of some points in the anatomy of the adult.

The animals live at a depth of about eight inches below the surface of the sand, and are generally to be found with their bodies coiled in an even corkscrew-like spiral. The proboscis and anterior part of the branchial region are usually vertical, and the portion of the body posterior to the generative tract, which is about 6—9 inches long, is also, as a rule, not spirally disposed, but can be moved up and down a vertical shaft in the sand opening to the surface. By this shaft fecal matters, consisting mainly of sand and mucus, are extruded.

In this manner very characteristic conical coiled casts are thrown up, like that of the earthworm, the section of the coil being, however, elliptical. The whole body is from 8 inches to 1 foot in length.

**Fertilisation and Segmentation.**—The animals are diœcious. The ovaries lie in an irregular band along the dorso-lateral aspect of the animal, and the testes in the male occupy a similar position. The dehiscence appears to take place by a rupture of the body-wall in both sexes. When the eggs are laid they are small, ovoid, very opaque bodies, of a greyish

yellow colour, about  $\frac{3}{8}$  mm. in length; enclosed in an elastic, close-fitting, transparent eggshell (fig. 1).

The spermatozoa dehisce in lobate spermatophoric masses they have spherical heads, and short flagelliform tails, with which they swim actively.

No attempt at artificial fertilisation was successful, for though the spermatozoa attached themselves to the egg the result was an abnormal segmentation; the ovum dividing rapidly into a large number of isolated spherules, which subsequently died. It was, therefore, necessary to obtain naturally fertilised ova. These were to be found without much difficulty in considerable numbers deposited in the muddy sand which the adults inhabit.

After fertilisation the appearance of the egg alters; the ovum itself becomes spherical, and the eggshell increases in size, being separated from its protoplasmic contents by a considerable space (fig. 2). It is not at all clear by what processes this change is accomplished, since the shell of the ovarian ovum is a tough and apparently structureless membrane, which does not appear to alter in character after fertilisation.

The first furrow is formed in a median plane, dividing the ovum into two equal parts. It passes to a considerable depth (fig. 3). With regard to the subsequent segmentation I have no certain observations; for though some of the ova divided into four and eight nearly equal parts, these were obtained by artificial fertilisation, and the process of division was afterwards continued in an entirely abnormal manner as mentioned above.

Judging, however, from the characters of the blastosphere (figs. 4 and 18), and from the fact that yolk granules are uniformly distributed through the whole tissue, there can be little doubt that the segmentation is regular and complete.

In the next stage which was found a spherical blastosphere was formed (fig. 4). The walls of this were opaque, but the outlines of the cells composing them could be faintly distinguished in a surface view.

Subsequent External Changes.—Before proceeding to describe the internal structure of this and the following stages it will perhaps be best to describe the changes which

occur in the external appearance of the embryo from this point until the form shown in fig. 16 is reached.

For convenience of reference I shall allude to the Stage shown in fig. 7 as Stage A; in fig. 9 as Stage B; in fig. 10 as Stage C; in fig. 11 as Stage D; in fig. 13 as Stage E; in fig. 14 as Stage F; in fig. 15 as Stage G; in figs. 16 and 17 as Stage H.

The Gastrula.—The blastosphere is at first spherical. It next assumes an elliptical form and gradually becomes flattened on one side (figs. 18 and 19). The flattened side next becomes concave and is rapidly invaginated, forming a cup-shaped gastrula (figs. 5, 20, and 21). This gastrula is radially symmetrical and the blastopore is still circular. The edges of the blastopore then approximate, and during this process the embryo grows unequally, causing one of its axes to elongate slightly (figs. 6 and 7). As a result of these changes the blastoporic aperture forms a very short slit, placed in a depression lying rather towards the pole which afterwards forms the posterior end of the animal. As the closing of the blastopore proceeds the whole surface of the body becomes covered with very minute cilia and a ring of larger cilia develops round the blastopore which is placed in a slightly eccentric position within it (figs. 7 and 8).

Stage A is thus reached. When the cilia appear, the larva rotates in the eggshell on the blastoporic axis in the direction of the hands of a watch.

Stage B is attained merely by an elongation of the axis at right angles to the plane of the ring of cilia, whose position makes the determination of the approximate position of the blastopore possible throughout larval life (fig. 9). An examination of a series of sections through a larva of this stage shows that the blastopore has closed.

The changes from Stages B to F occupy about fifteen hours. In Stage C the long axis is still more marked.

In Stage D (figs. 11 and 12) the body is further elongated and slightly flattened, the flat surfaces being afterwards shown to be dorsal and ventral respectively. A transverse constriction

has appeared (fig. 12, g.) dividing the body into two nearly equal parts. Subsequent changes show that this groove marks off the region which is to form the proboscis from the rest of the body.

As this groove increases in depth a second groove is formed behind it (fig. 13, c. g.). The area between these two grooves forms the collar. The proportions of the body have also altered considerably; the length of the proboscis being now only about a third of the total length of the animal. At this stage arises also a tuft of long flagelliform cilia at the apex of the proboscis. No eye-spots are present, however, at this or at any subsequent stage.

The animal remains in this condition for some hours and is generally hatched without the occurrence of any further alteration. The time of hatching is, however, quite irregular. Larvæ may frequently be found swimming freely whose organisation is not much in advance of Stage C, and on the other hand, I have seen them in the condition of Stage G still enclosed in the eggshell.

While in the eggshell from Stage C onwards the larva swims about very rapidly, rubbing the membranous shell with its anterior end until it gives way, and the animal escapes. On leaving the egg it does not swim at the surface as pelagic larvæ do, but creeps about in the mud, burrowing with its proboscis, in the walls of which muscle fibres soon appear (v. infra), and also propelling itself by means of its ciliated band. If placed in a beaker of water it sinks to the bottom at once. Two specimens of Stage H were, however, taken on one occasion in the surface-net, but from the molluscs and other creatures present with them, it was clear that the net had been allowed to drag along the bottom. As the water was never more than four feet deep at high water this was easily possible, and occurred continually at Hampton.

Stage F.—The next feature of importance which generally occurred shortly after hatching, was the appearance of a longitudinal groove in the middle dorsal line of the collar (fig. 14, n g). This groove is a temporary structure, only lasting



two or three hours, as will be shown when the internal development is described. The appearance of this groove occurs at the time of the delamination of the dorsal nerve-cord in this region of the body.

Stages G and H.—Simultaneously with the disappearance of this neural groove, two pores are to be seen perforating the skin behind the collar, being placed in a dorso-lateral position, one on each side of the middle line. These pores in which long cilia may be seen working, constitute the first pair of gill slits (fig. 15, *g. s.*). In this stage the collar has become much shorter, and the cilia of the band are so densely crowded as to give an appearance of a thickened ring in preserved specimens. After the formation of this pair of gill slits no further external change of importance is to be noted in any of the oldest larvæ which I found. The proboscis becomes longer and more muscular, and the body increases somewhat in size; the region of the body behind the ring of cilia especially elongates. The ring of cilia may, moreover, in a profile view, be seen to lie no longer in a truly transverse direction, but rather obliquely with its dorsal limb anterior to the ventral portion (fig. 17). The body is usually somewhat bent upon the ventral surface while the animal swims. This feature is usually exaggerated after death, and in consequence from the dorsal side the whole length of the animal is not seen (fig. 16).

The mouth is formed in Stage F as a minute pore placed on the ventral surface in the groove which divides the proboscis from the collar; from its position and small size it is not visible when the animal is examined as a whole. The formation of the anus occurs rather later by a perforation of the skin at the posterior end; it also is best seen in sections. In position it is approximately coincident with the point at which the blastopore finally closed.

I have not hitherto been able to obtain any older larvæ whose external features differ essentially from Stage H. Judging from the fact that at this stage a considerable increase in size takes place, and from the commencement of several important internal structures at this time, it seems probable that the

animal passes a prolonged period in this condition without any external modification, but this, of course, is quite uncertain. The larvæ were all found between July 20th and September 6th, and I am in hopes that at some other season of the year it may be possible to find the remaining stages between H and the adult condition.

Very considerable structural modifications must, of course, occur before such a larva as Stage H can assume the external form of the parent. These changes must consist chiefly in a great increase in the length of the body, in the number of the gill slits, &c. ; but probably the point of greatest interest lies in the subsequent development of the collar, which in the adult presents an appearance considerably different from that of the same structure in this larva, both in position and extent.

Internal Structure.—Having dealt with the external appearances, I will now describe in detail the internal structure from the blastosphère to Stage E.

An account of the formation of the mouth and nerve-cord I also introduced into this paper, but I propose to leave the description of the other changes in Stages F, G, and H, together with an account of some points in the anatomy of the adult, to be given in a subsequent paper which I hope to prepare shortly.

The characters of the blastosphère are shown in fig. 8. The walls are formed of a layer of single cells enclosing a segmentative cavity. This individual has already become slightly flattened, having previously been spherical as shown in fig. 4. Simultaneously with this compression a differentiation has commenced between the cells which are to form the epiblast (*E*) and those which will be invaginated to constitute the hypoblast (*H*). The former are smaller and less granular than the latter, which are large and contain many yolk particles in their peripheral ends. The central ends of the hypoblastic cells have a characteristic amœboid appearance ; between these two parts of the cell the large dotted nucleus is placed.

Between the epiblastic and hypoblastic portions the walls are composed of indifferent cells.

The segmentation cavity (*s. c.*) is a large empty space; and neither at this, nor at any subsequent period of development do any mesenchyme cells appear in it, the mesoblast arising entirely from the walls of the Archenteron, as will be afterwards described.

By a progressive differentiation the plano-convex form shown in fig. 19 is reached. At this period the disparity in size between the cells of the two regions is still more marked, and no part of the wall is composed of indifferent cells.

Figs. 20 and 21 represent the appearances seen in transverse sections of rather older Gastrulæ. No histological change of importance has taken place. In fig. 22 the fusion of the layers which accompanies the closing of the blastopore is shown. This region of fusion is seen in a series of sections to be somewhat longer in one direction than in the other, conformably with the external characters of Stage A.

In Stage B the blastoporic fusion still persists, but the hypoblast is nearly separated from the epiblast, only remaining connected with it for a very small area, which marks the point at which the blastopore finally closed. When Stage E is reached this area is still further reduced, but can be traced until Stage D.

In a section through the anterior end of this stage, the epiblast is seen to be composed of large cuneiform cells containing granules, whose outer ends are covered with very minute cilia. Between their internal ends spaces are shown, which are no doubt due to contraction of the protoplasm caused by reagents; similar spaces are also observable between the cells of the hypoblast. At the front end of this larva these latter take a more amœboid character than in the middle and posterior regions (fig. 25, *H'*).

The epiblast is closely applied to the hypoblast in the anterior half of the larva, causing the segmentation cavity to be obliterated. The hypoblast of the middle region is seen to be more columnar in character than that of the anterior region, while the other appearances are the same (figs. 23 and 25).

A section through the blastoporic tract of the same larva is given in fig. 24. The segmentation cavity is here still large, and the walls of the archenteron are found fused with the epiblast at a point at one side of the larva which is otherwise a radially symmetrical, double-walled cylinder. At this point the archenteric cavity also slopes to an angle which persists after the complete separation of the walls from the epiblast. By the persistence of this appearance the point at which the closure of the blastopore is completed can be identified as being nearer to the dorsal surface of the animal, being at the junction of the dorsal and posterior surfaces. The archenteric cavity contains a quantity of coagulum at this and subsequent stages.

The transverse constriction which now arises (fig. 11, *g*) lies at the junction of the columnar portion of the hypoblast with the more amœboid part, mentioned above as being found in the anterior end. It is, at first, merely a groove in the epiblast, which coincides with a similar depression in the hypoblast. As will presently be described, the walls of that part of the archenteron which lies in front of this groove become constricted off to form the unpaired anterior body cavity; and the commencement of the consequent histological differentiation may be already perceived in the more amœboid character of the hypoblast cells in this region (fig. 25, *H'*).

The internal relations of the parts between Stages C and D are shown in fig. 25, which is constructed from the same series of transverse sections from which figs. 23 and 24 were taken.

In Stage D the hypoblast separates completely from the epiblast, and the appearances to be described in Stage E begin to be present.

Stage E—Formation of the Mesoblast.—Leaving the larva in the form of the closed, two-walled cylinder described above, whose cavity has already begun to become constricted into two parts, the anterior being lined by somewhat amœboid, hypoblastic cells, and the posterior by regular, columnar hypoblast, a description of the changes to be seen in Stage E will be given in detail.



Figs. 26—34 are drawn from a series of sections through a larva at this stage, of which fig. 13 is an external view. It will be remembered that the body now consists of three regions. (1) An anterior lobe which on the formation of the mouth will be seen to be præoral; (2) a narrow area between two grooves, which will be afterwards spoken of as the collar; and (3) the rest of the body, which will be alluded to as the trunk; this trunk portion is secondarily divided into an anterior and posterior region by the transverse band of cilia.

The dorsal and ventral surfaces may now be distinguished in section, as a thickening of the epiblast is already to be found in the collar on one surface. This structure, which subsequently forms part of the central nervous system, marks the middle dorsal line. The body is now elliptical in section, the long axis of the ellipse being horizontal.

The epiblast now consists of small elongated cells, which are arranged from two to three deep, bearing minute cilia on their peripheral ends. This layer is of uniform thickness and constitution over the whole body, with the exception of the small linear area in the middle dorsal line of the collar, mentioned above. In this region it is somewhat thicker, but no differentiation has occurred among its cells. Moreover, the cells which carry the transverse band of cilia are slightly larger and more columnar than the rest.

The detailed structure of these epiblastic cells is shown in fig. 31, *a*, *E*. They are small, dense cells, containing a considerable quantity of granules, which stain deeply with reagents. The nucleus is proportionally large, and also contains granules.

The mesoblast arises at this period of development. It is formed directly by differentiations of cells belonging to the archenteron. These differentiations occur in five regions. The first comprises a median and primitively unpaired tract in the anterior end, which forms the lining of the body cavity of the præoral lobe. Behind this anterior body cavity a pair of mesoblastic differentiations occur in the region of the collar, constituting lateral outgrowths of the archenteric walls, each con-

taining a cavity which communicates directly with the cavity of the archenteron. Behind these, again, is another pair of regular archenteric diverticula, in the region of the trunk. This mode of origin of the mesoblast, which will be made clear by the diagram (fig. 40), will now be described in detail. It will, however, be perhaps simplest to describe all the remaining parts, beginning at the anterior end of the animal, and proceeding backwards.

### 1. The Anterior Body Cavity.

On reference to the longitudinal section of the larva between Stages C and D (fig. 25) it is seen that the cells lining the front of the archenteric cavity had a character different from that of the remaining hypoblast. When Stage E is reached this differentiation has greatly increased, and the cells which line this anterior region may now be seen to have an entirely peculiar appearance. They are still closely applied to the epiblast (fig. 26, *M'*) at one end.

This peripheral end is broad, and is continued into a narrower portion, which again dilates to form the round head of the cell, which projects into the cavity of the præoral lobe. These round central extremities, in which the nuclei are generally to be found, are continually budding off round cells into the cavity in which they lie. At this period, however, this process of proliferation is only commencing, and the cavity of the præoral lobe is therefore lined by a layer of cells, which is for the most part only one cell deep.

In the peripheral ends of these cells, which, as will be seen hereafter, are destined to form part of the mesoblast, an appearance may be noted which, together with the absence of granules in this part of the cell, gives it a look of semi-fluidity. This appearance is characteristic of a large part of the hypoblastic and mesoblastic tissue in a larva of this age, and disappears about the time at which the mouth is formed.

Whether it is actually due to the presence of fluid contents in the cells or not I cannot say; since, however, a considerable increase in the size of the body occurs before the animal leaves

the egg, and in the absence of a mouth, it is tolerably certain that this growth must be due to the taking in of water concomitantly with the using up of the yolk particles, deposited especially in the hypoblastic tissues.

An attempt is made to indicate this constitution of the cells where it occurs by a blurred shading in the figures.

The nuclei of these cells, and of all the cells of this layer, are irregular in shape.

The cavity, lined by these cells, which will be spoken of as the anterior body cavity (fig. 26, *bc*, 1), still communicates with the original cavity of the archenteron, and the layer of cells which forms its inner wall is still directly continuous with the hypoblast itself. This continuity is shown in fig. 27.

The gut here projects into the anterior body cavity as a tube the end of which is obliquely truncated (fig. 35), so that the ventral lip projects further forwards than the dorsal. In a section taken behind that shown in fig. 27 the archenteron is therefore seen as an elliptical structure, lying inside the anterior body cavity, complete on its dorsal side.

The free edges of the hypoblastic tube are continuous with the body cavity epithelium, which is reflected backwards from it, owing to the backward prolongation of the cavity itself. This backward prolongation of the anterior body cavity is not of the same extent on all sides. Ventrally to the gut, it is very slight, and occurs in very few sections. It appears in fig. 27 as a small space below the ventral wall of the gut. On the dorsal, and especially on the lateral aspects of the body, the posterior parts of the anterior mesoblast are more conspicuous, and will be subsequently shown to be of considerable importance.

Now, since the anterior body cavity is continued behind the end of the gut on all sides excepting the ventral, it is crescentic in shape, the concavity being directed downwards. This appearance exists only for a short distance. Behind it the continuity across the dorsal surface ceases, and the mesoblast exists as a pair of small, hollow cavities at the dorso-lateral sides of the gut, which is here much more fully developed,

occupying most of the space enclosed by the epiblast. Still further backward the cavities in these two mesoblastic tracts close up, and their walls are continued for a short distance as two solid cords of cells, and then disappear.

The mesoblast of the anterior body cavity is, therefore, formed directly from the walls of the hypoblast, which occupied the same situation. It is separated off from it by a process of constriction in the region of the external groove, dividing the proboscis from the collar (fig. 13, *g*). While this process of constriction is being carried out, the pouch of mesoblast grows backwards, surrounding the gut except on the ventral surface, but especially forming the hollow horns (fig. 28, *r. M'*, *l. M'*), lying in a horizontal position, one on each side of the gut.

These relations are made clear by the diagram (fig. 40), and the continuity of the gut with this anterior mesoblastic wall is shown best in longitudinal, median, vertical section (fig. 35. This section is taken through a larva slightly younger than Stage E.)

The anterior body cavity is not completely constricted off from the gut until a later period.

In this larva the hypoblast itself is composed of large cells with rounded outlines, containing elongated nuclei of variable shape. Some of these cells are granular, while others have the fluid appearance described above. In the regions which are not concerned in the formation of the mesoblast, these cells lie in two to four irregular layers. A section through the region of the posterior horns of the walls of the anterior body cavity is shown in fig. 28. It is in about this position that the mouth is eventually formed on the ventral surface. The mesoblastic tissue of the anterior body cavity is seen as two lenticular masses of large cells, lying on each side of the archenteron in the dorso-lateral regions of the body, their cavities being closed.

The Nervous System.—In the dorsal middle line of this region a slight thickening of the epiblast is visible which is the rudiment of the central nervous system. As yet, however, no further development of it is present (fig. 28), and it merely



forms a slight inwardly projecting prominence in transverse section.

## 2. The Middle and Posterior Body Cavities.

A section through the tract behind the anterior mesoblast, and in front of the middle body cavities, is shown in fig. 29.

The form of the body is elliptical in section, and the epiblast does not differ from that previously described. No rudiment of the nervous system is as yet found as far back as this. The hypoblast is here closely applied to the epiblast throughout its circuit, no space being left between them. The cells of the hypoblast are here from two to three deep, and have the constitution described already. No other structures are present in this region; this part of the body consists, therefore, merely of a tube, with two walls placed in apposition. This simple, two-walled condition only extends for a very short distance, and transverse sections taken immediately behind that shown in fig. 29 exhibit a narrow split-like cavity in the wall of the archenteron on either side of the body. This pair of cavities is bounded on the inner sides by the cells forming the wall of the gut, and the external boundary is made up of a single layer of cells continuous dorsally and ventrally with the hypoblast. These two cavities are the middle pair of body cavities, and their walls constitute median mesoblastic tracts. Immediately behind the point at which they first appear, their cavities may be seen to be connected with that of the archenteron by means of two small pores rather below the middle horizontal line (fig 30, for. 2). This connection is only visible in very few of the larvæ, and may possibly be due to the action of reagents. Since, however, the middle mesoblastic tracts in *Tornaria* are said to be archenteric diverticula (Spengel, &c.), it seems more likely that the rarity of their occurrence is due to the shortness of the time for which they are present.

An examination of the succeeding sections shows that these foramina are placed almost at the anterior end of the second body cavities, and that their principal extension is therefore posterior to their openings. A section through these cavities

behind the openings is figured (fig. 31). They here extend along nearly the whole vertical depth of the archenteron. The cells forming their outer wall are very granular, and project in an irregular way into the cavity which they enclose. The inner wall is formed of cells still in connection with the hypoblast, whose contour is also rounded and irregular. As yet they only differ slightly from the rest of the hypoblast, being smaller and somewhat more granular. The minute structure of the layers in this region is shown in Fig. 31, *a*. The cavities extend from the anterior groove to the posterior one,—that is to say, throughout the length of the “collar” at this stage.

Behind them the archenteric wall is simple and the body is a two-walled elliptical cylinder, presenting nearly the same appearances which were described as occurring immediately in front of the collar. The archenteric walls are, however, somewhat thicker. This arrangement only occurs for a very short distance, and is continued as far backwards as the third mesoblastic region, which begins in front of the transverse band and cilia. In the anterior end of this area the hypoblast is split on each side, thus enclosing a pair of cavities similar in appearance to those of the second mesoblastic region, but differing from them in having a greater horizontal extent. The cavity of the archenteron is therefore reduced in this part of the body. These mesoblastic pouches open by large foramina (fig. 34) into the lumen of the gut; these openings occur in the posterior third of their extent. As will be seen in fig. 33 the outer walls of these two cavities do not continue the curve of the hypoblast, but spring from it, bending outwards, consequently forming a pair of archenteric diverticula.

In transverse section through the communicating foramina the ventral wall of the archenteron is thicker than the dorsal. Posteriorly this thickening increases, and, meeting the dorsal wall, closes up the lumen of the archenteron. As previously stated, the anus is not found until a later stage is reached. This completes the account of the anatomy of the larva at this period.

It may be useful to sum up the principal points of internal structure which are to be observed in Stage E. Of these the most important features are :—(1) The commencement of the nervous system as a median dorsal thickening of the epiblast in the region of the collar; and (2) the formation of the mesoblast. Briefly to recapitulate the latter process, it consists of (*a*) the constricting off of the anterior portion of the archenteric cavity from the remainder to form a single median impaired mesoblastic pouch, which secondarily sends back a pair of hollow outgrowths placed one on either side of the gut; (*β*) the formation of a pair of cavities in the archenteric wall in the region of the collar whose lumina open into the lumen of the gut; (*γ*) the appearance of a pair of archenteric diverticula in the posterior region of the trunk.

It will therefore be observed that the process of formation of the first body cavity is constituted by a direct specialisation of the hypoblastic wall, and that while the posterior pair of mesoblastic somites are actual archenteric diverticula, the origin of the anterior pair partakes of the characters both of archenteric diverticula and of delamination from the hypoblast. These two modes of origin of the mesoblastic somites are, of course, essentially the same, differing from each other merely in degree. This case is perhaps interesting as affording an illustration of these two methods both occurring in the same animal, and the combination of the two processes in the case of the middle somites is especially noteworthy as representing the last stage of the phylogenetic transition from hollow archenteric outgrowths to mere plates delaminated from the hypoblast.

Fig. 35 represents a longitudinal vertical section through a larva slightly before Stage E is reached, in which the nervous system is not yet formed even in a rudimentary condition. As the plane of the section passes through the middle line the middle and posterior portions of the mesoblast are not shown. The relations of these to the regions of the body are illustrated by fig. 40, which is a diagram of a horizontal median section of such a larva.

The Separation of the Mesoblast.—The next changes of

importance consist in the closing off of these five mesoblastic pouches. The foramina opening from the archenteron to the middle pair are the first to become obliterated by the coalescence of the hypoblast surrounding them. Shortly after this has occurred the layers of cells forming their inner wall segregate themselves from the archenteric wall.

This process leads to the formation of a pair of closed sacs lying one on each side of the archenteron in the region of the collar.

While the middle pair are separating, the process of closure is also completed between the anterior body cavity and the archenteron by the fusion of the margins of the hypoblastic tube previously described as projecting into the anterior body cavity. The cells composing this tube then retreat backwards, and cease to project beyond the limit of the general anterior wall of the gut. The resulting condition is shown in fig. 36, which is drawn from a longitudinal section which does not pass quite through the middle dorsal line, and is nearly parallel to the longitudinal vertical plane of the body. (Owing to this fact the nervous system is not shown.)

In this section the cells forming the posterior wall of the anterior body cavity form a layer in apposition with the outer ends of the cells constituting the anterior wall of the gut in the region at which the communication between the two cavities previously existed (vide fig. 35). The anterior wall of the gut is here composed by a single layer of cells.

The posterior mesoblastic pouches are shut off from the archenteron in a manner similar to that of the middle pair.

On the completion of the separation of these various portions of mesoblast from the gut the cells of their walls change their primitive character.

Subdividing rapidly they become much smaller, assuming, except in the case of those of the anterior body cavity, the appearance of ordinary peritoneal cells. Those of the anterior body cavity proliferate rapidly in the manner mentioned above, especially in the dorsal and ventral middle lines, forming a pair of large masses of spherical cells in these situations.



Some of the cells lying on the external sides of these masses early become converted into muscular fibres before Stage F is reached (fig. 36, *m. f.*). A full account of these and the subsequent changes occurring in the anatomy of the præoral lobe will, I hope, be subsequently given, together with a description of the structure of the later stages of the larvæ.

Further Development, Stages F, &c.—With regard to the further development of the animal, I propose on this occasion to speak only of the formation of the mouth, and of the central nervous system.

The Mouth.—The mouth is found at the end of Stage F as a small perforation in the ventral middle line lying in the groove which separates the præoral lobe from the collar. At this point the wall of the hypoblast forms a short, downwardly directed diverticulum; this is partially shown in fig. 36. Its outer wall comes into close contact with the epiblast and then fuses with it, a perforation being formed through these coalesced tissues. There is therefore no regular stomodæal invagination. As mentioned above, when first formed the mouth is very minute and quite indistinguishable in a surface view.

Fig. 37 represents a transverse section through the mouth at a rather later stage. The body cavities occurring on either side of it are portions of the middle pair.

The Nervous System.—In Stage E a slight thickening of the epiblast could be distinguished in the dorsal middle line of the collar, having a short, longitudinal extent. It may be remembered that in Stage F a slight groove was visible in this position, which existed for a short time and then disappeared (fig. 14, *n. g.*).

A series of sections through a larva in which this groove is still visible exhibits the following arrangement in the epiblast of the collar. In the extreme front portion of the collar the epiblast is slightly thickened in the dorsal middle line, presenting an appearance very like that seen in the same region in Stage E. A depression is also visible on the dorsal surface at the point where the neural groove is cut across. In the middle

third of the collar this thickening is much more marked, and is formed by a cord of columnar cells whose characters differ from those of the rest of the epiblast (fig. 38, *n. s.*). These cells are rather larger and somewhat pyramidal in section, their bases forming the inner border of the epiblast in this region. Their apices converge towards the centre of the cord. By a continuation of this process of convergence this portion of the epiblast in the posterior third of the collar segregates itself from the skin, forming an apparently solid rod of cells immediately below the epiblast but detached from it in the dorsal middle line of the collar (fig. 39, *n. c.*) This separation from the skin is extended backwards and forwards along the whole length of the collar, but is never completed at either end of it, where the continuity persists throughout life.

As has been stated this nerve cord is at this stage apparently solid; but as may be seen upon examination of the same structure in the adult it eventually possesses a distinct lumen for a great part of its course.

[In fig. 39 it will be observed that a transverse section cuts both the middle and posterior body cavities. This is due to a forward growth of the posterior body cavities on the ventral side of the middle pair. As a result of this growth the septa dividing the two cavities come to lie obliquely, instead of being in a transverse place.]

As it is not proposed on the present occasion to proceed beyond this point in the account of the development I will now briefly recapitulate the chief facts in the history of the larva.

Recapitulation.—The eggs are elliptical and opaque, being fertilised outside the body. After impregnation they divide into two, the subsequent segmentation being probably regular and complete. Segmentation results in the formation of a hollow blastosphere, enclosing an empty segmentation cavity. One side of this blastosphere is next invaginated to form the hypoblast, thereby constituting a simple hemispherical gastrula. The blastopore closes completely; the point of closure being placed at the middle dorsal edge of the posterior surface. At

this period a posterior transverse ring of cilia is found. The body elongates, and becomes marked out into regions, by the appearance, first of an anterior groove situated nearly in the middle line, and secondly of a posterior groove shortly behind it. The area in front of these grooves is præoral, and is destined to form the proboscis, while the region between them constitutes the collar.

The invaginated hypoblast at first forms a simple lining to the cylindrical body. That portion of it which lies in front of the anterior groove then segments off from the rest, forming an anterior unpaired body cavity. This cavity sends back a horn for a short distance on either side of the gut.

In the region of the collar a pair of splits occur in the hypoblastic walls, whose cavities open into the archenteron. The cells forming their walls then separate themselves from the remaining hypoblast as a pair of closed pouches placed symmetrically, one on each side of the body.

A pair of archenteric diverticula are also formed in the region of the trunk, which, on losing their connection with the gut, persist as another similar form of body cavities.

The nervous system is formed by a segregation of epiblastic cells in the dorsal middle line of the collar, forming a cord lying immediately beneath the skin, continuous with it at both ends of its course.

The mouth is a small pore in the ventral middle line placed in the anterior transverse groove.

The larva is always opaque, and on being hatched creeps about in the muddy sand which the parents inhabit, at no time leading a free life at the surface comparable with that of *Tornaria*.

I hope shortly to describe the remaining structures existing at Stage F, and to give some account of the older larvæ, and of certain points in the anatomy of the adult.

It may, perhaps, be convenient briefly to allude to some of the latter which are of value in interpreting the facts already given. Of these, three are of especial importance.

1. The nervous system (Spengel) in the adult is made

up of four parts: a dorsal and ventral cord lying in the skin in the dorsal and middle lines respectively, connected with each other by a periœsophageal ring in the posterior fold of the collar; a continuation of the dorsal nerve through the collar, first as a solid cord, and afterwards as a hollow tube running in the body, separated from the skin by mesoblastic tissues; this collar portion being continuous with the skin at both ends; a continuation of the dorsal cord on to the proboscis, at the base of which it forms a considerable concentration, forming a ring in connection with the skin round the base of the proboscis stalk; lastly, a plexus of nerve-fibres over the whole of the body in close connection with the skin, which is particularly well developed on the proboscis.

2. The Proboscis-pore is a small ciliated opening into the body cavity of the proboscis, situated in the middle dorsal line in the region of the proboscis stalk. It opens through the thickest part of that concentration of the nervous system which encircles this part of the body (Spengel, &c.).

As will be afterwards shown, when the anatomy of the older stages is treated of, this pore leads into a small chamber lined with ciliated columnar cells. This chamber is continuous with that horn of the anterior body cavity which lay originally on the left side of the gut. It thus communicates with the general tissue spaces of the proboscis. The right posterior horn never becomes connected with the exterior.

3. In the adult a forwardly-directed diverticulum opens into the alimentary canal in the dorsal middle line, a little behind the mouth. For the chief part of its course it lies in the proboscis (Spengel).

It consists of large vacuolated cells, whose structure is somewhat peculiar, and bears a strong resemblance to the notochordal tissue of a young Elasmobranch.

The lumen of this diverticulum is large posteriorly, and anteriorly is almost entirely obliterated. This rod of tissue is the supporting structure of the proboscis.

It arises as a forward growth of the hypoblast at Stage G, in a manner to be afterwards described.



Though a detailed comparison or general discussion of the significance of these developmental facts cannot, of course, be attempted until the later stages have been described, it may perhaps be advisable to point out the chief features of difference between the larvæ just described and *Tornaria*.

In the first place it will be seen that at no stage has this larva any superficial resemblance whatever to a *Tornaria* possessing a longitudinal posterior band of cilia. This fact is the more remarkable, as the adult appears closely to resemble *B. Kowalevskii*, which is described by Agassiz as passing through a *Tornaria* stage.

At Stage H, however, the general contour of the body is very like that of the late stage of *Tornaria*, and especially that species described by Metschnikoff, in which a single pair of gill slits is present. (In the *Tornaria* figured by Agassiz, in this condition, four gill slits are already formed.)

Now *Tornaria* (Metschnikoff, &c.) is a transparent larva, possessing a præoral band of cilia; a longitudinal band of cilia, and one or more transverse posterior bands of cilia, also a nearly median water-vascular, archenteric diverticulum, opening to the exterior a little to the left of the dorsal middle line. It also has an apical epiblastic thickening, bearing a tuft of cilia and a pair of eye-spots, from which a contractile string passes to the inner end of the water-vessel.

On the other hand, this larva is opaque; it has no præoral or longitudinal postoral bands of cilia, water-vascular system, eye-spots, or contractile string, thereby differing markedly from *Tornaria*.

It resembles it in the possession of a transverse band of cilia and an apical tuft of cilia.

The opacity of this larva is however due to the presence of food yolk in its tissues, and most of these points of divergence are more or less such as might be expected to result from this fact, consisting, as they do, chiefly in the absence of such a complete apparatus for locomotion and of the sense organs; for it is clear that an animal which passes a large part of its larval life enclosed in an eggshell, and on emerging from it creeps in the

mud, has less need of bands of cilia and organs of sense than a larva which leads a free existence at the surface.

Less intelligible are the absence of a water-vessel and of a contractile string. With regard to the former, Spengel states that in *Tornaria* it eventually forms the body cavity of the præoral lobe and its pore persists as the proboscis pore. It becomes, therefore, certain that the anterior body cavity of the opaque larva, which has a similar fate, is homologous with the water-vessel of *Tornaria*. Moreover, in *Tornaria* the water-vessel opens to the left of the middle line, while in this larva the external opening when it appears is connected with the left posterior horn of the cavity. The origin of the middle and posterior pair of mesoblastic sacs is similar in the two larvæ, and their eventual disposition in the opaque larva corresponds generally with the fate described by Spengel for the two pairs of archenteric diverticula in *Tornaria*.

Since Metschnikoff has recently published a paper in which he proposes to class *Balanoglossus* together with the Echinodermata in a common class "Ambulacraria," and since this proceeding is mainly supported by arguments adduced from a comparison of *Tornaria* with Echinoderm larvæ in general, and with a typical Asteroid larva in particular, it will be necessary to see what new light the existence of a second type of *Balanoglossus* larvæ throws on this thesis. Until the later development of the opaque larva is known it is impossible to make any such comparison, but it is perhaps worth pointing out that while this larva differs from *Tornaria* in many points it happens that all of these points (if we except the presence of eyespots) are those in which *Tornaria* resembles an Asteroid larva. Nevertheless, most of these features, the præoral and longitudinal postoral bands of cilia, &c., are very possibly purely secondary structures whose presence is correlated with a pelagic habit of life. If this is true, however, they could not avail either to connect or to separate *Tornaria* from the Asteroid larvæ, and the existence of an opaque *Balanoglossus* larva which lacks them would have no significance in settling this question.

The absence of any proper water vessel and contractile

string is, however, of much more importance, and a full discussion of the meaning of it must be deferred. But in comparing the modified anterior mesoblastic sac in the opaque larva with the water vessel of the larvæ of Asteroids, &c., one point is obviously remarkable and may conceivably be of some morphological value, viz. that while in Asteroid larvæ the water vessel is developed always from the left primitive archenteric diverticulum so in *Tornaria* the original evagination from the gut to the exterior is on the left side of the body (Götte). In the larva and adult of *Balanoglossus* (sp. incert.), and in the adult of *B. minutus* the permanent opening communicates with the left posterior horn of the anterior body cavity the cells of which become columnar, while those of the right horn have the structure of ordinary connective tissue. The origin of the water vessels from the left vesicle is also true generally speaking for Echinoidea and Ophiuroidea. It would therefore appear to be of some morphological importance. Upon these and other points in the relationship of *Balanoglossus* to other forms, the later development may be expected to afford some information.

There is one more comparison which may, I think, be shortly alluded to since it is suggested by even a superficial examination of the early stages of this opaque larva.

Since *Balanoglossus* possesses gill slits which are not comparable with any structures present in animals outside the Chordata, it appears *prima facie* as worthy of consideration whether the presence of these structures may not point to a common origin.

Now leaving this question aside for the present, I would suggest that a very striking similarity does exist between the general history of the early development of this larva and that described by Hatschek for *Amphioxus*, this resemblance being more particularly strong in the situation and mode of origin of (1) the central nervous system and of (2) the mesoblastic somites.

For according to Hatschek's account of its origin, the nervous system in *Amphioxus* differs from the central nervous system in

this larva, mainly in its extent, and in the fact that in *Amphioxus* it encloses a neurenteric canal. Now, with regard to the second of these differences, from a consideration of the position of the blastopore in my larva (fig. 25), it is clearly only necessary to imagine the invagination of the dorsal nerve cord to have been extended along the back (instead of being confined to the region of the collar) in order to reproduce the condition which is found in *Amphioxus*; for the dorsal nerve cord in the adult *Balanoglossus* is as a matter of fact continued into this region, though in connection with the skin.

With regard to the origin of the mesoblast, the following is the arrangement in *Amphioxus* (Hatschek). It is formed anteriorly by a primitively unpaired pouch of hypoblast, which is continued into two posterior horns; this anterior pouch is followed by a great number of paired pouches lying on each side of the body which are constricted off from the gut. The anterior pouch is the last to close. As it does so, its cavity divides into a pair of pouches, lying one on the right hand, the other on the left. Of these the cells of the left become columnar and ciliated, and its cavity opens to the exterior, while the tissue of the right pouch becomes flattened epithelium, lining the body cavity of the head. On the other hand, in the larva now described the mesoblast is formed from an anterior archenteric pouch with two posterior horns, followed by only two pairs of pouches. Of the two incompletely separated divisions of the anterior cavity, that which lies on the left side becomes lined by ciliated columnar epithelium, and opens to the exterior, while the right hand one forms connective tissue. The origin of the mesoblast in *Amphioxus* differs, therefore, in being accomplished by a great number of paired posterior pouches instead of by two; and in the fact that the division between the right and left parts of the anterior pouch is completed instead of being partial. There appears, therefore, to be a general agreement in the early development of these two animals which holds good even in the remarkable asymmetry above described.

At first sight it seems likely that these points of resem-



blance are more than superficial, especially when it is remembered that the adults of both animals possess essentially similar branchial structures which, beyond the Chordata, are otherwise without parallel in the animal kingdom. There are, of course, many and great difficulties which preclude any assumption of relationship between them, notably the absence of any regular notochord in *Balanoglossus*.

On this occasion it is not profitable to discuss these questions at greater length. When, however, the later development of this form has been described, it may perhaps be possible to arrive at more definite conclusions.

In closing this paper I have to thank Mr. Sedgwick and Mr. Weldon for rendering me much valuable assistance and advice in connection with it.

## EXPLANATION OF PLATES XVIII, XIX, XX, & XXI,

Illustrating Mr. William Bateson's paper on "The Early Stages in the Development of *Balanoglossus* (Sp. incert.)."

### *Complete List of Reference Letters.*

*a.* Anus. *A. c.* Archenteric cavity. *b. c.* 1, 2, and 3: first, second, and third body cavities respectively. *bl.* Blastopore. *bl. pl.* Blastoporic pole. *c.* Apical tuft of cilia. *c. g.* Groove separating the collar from the trunk. *cil.* Transverse band of cilia. *cl.* Collar. *D.* The dorsal side. *E.* Epiblast. *for.* 1, 2, 3: foramina, by which first, second, and third body cavities respectively communicate with the lumen of the gut. *g.* Groove separating proboscis from the collar. *g. s.* Gill slit. *H.* Hypoblast. *H'.* Hypoblast beginning to be differentiated to form the mesoblast of the præoral lobe. *H''.* *H'''.* Cells still forming part of wall of archenteron, but which are beginning to separate off as the inner walls of the second and third body cavities respectively. *l.* *M'.* Left posterior horn of anterior mesoblastic sac. (Vide note below.) *l. g.* Longitudinal ventral groove in gut. *m.* Mouth. *M'.* *M''.* *M'''.* Mesoblast of first, second, and third regions. *m. f.* Muscle fibres forming in the mesoblast of the præoral lobe. *n.* Commencing nervous system. *n. c.* Nerve

cord. *n. g.* Neural groove. *p.* Proboscis. *pt.* Point at which anterior body cavity closes. *r. M'*. Right posterior horn of anterior body cavity. (Vide note below.) *s. c.* Segmentation cavity. *sh.* Shell.

I have to thank Mr. H. A. Chapman for assisting me with the figures of the surface views.

Fig. 31*a* was very kindly drawn for me by Mr. W. F. R. Weldon.

The outlines were drawn with Zeiss's camera lucida.

Figs. 1—17.—Surface views of eggs and larvæ of successive stages. (Zeiss's Obj. A, oc. 2.)

FIG. 1.—Ovum just laid, unfertilized.

FIG. 2.—Fertilized egg.

FIG. 3.—Egg segmented into two by a median furrow. The eggshell is not afterwards represented.

FIG. 4.—Blastosphere, cell outlines visible.

FIG. 5.—Gastrula seen from blastoporic surface.

FIG. 6.—Later gastrula; blastopore contracted, seen from the side.

FIG. 7.—Closing gastrula, seen from the mouth; ciliated. (Stage A.)

FIG. 8.—The same seen from the side.

FIG. 9.—Larva shortly after the blastopore has closed, from side. (Stage B.)

FIG. 10.—Older larva. (Stage C.)

FIG. 11.—Stage D. (Drawn from a preserved specimen.)

FIG. 12.—Larva between Stages D and E.

FIG. 13.—Stage E.

FIG. 14.—Stage F.

FIG. 15.—Stage G, from the side.

FIG. 16.—Stage H, from dorsal surface. (Drawn from a preserved specimen.) Appears shortened, owing to ventral flexure.

FIG. 17.—Stage H, from the side.

FIG. 18.—Vertical section through centre of a late blastosphere. (Zeiss's Obj. D, oc. 2.)

FIG. 19.—Section through plano-convex blastosphere. (Obj. C, oc. 2.)

FIGS. 20 and 21.—Sections through the blastopore of gastrulæ in two stages. (Obj. C, oc. 2.)

FIG. 22.—Transverse section through a closed gastrula, between Stages A and B. (Obj. B, oc. 2.)

FIG. 23.—Transverse section through the anterior end of a larva, between

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[Note.—In interpreting those figures in which the right and left sides of the animal are indicated, it must be remembered that as the figures are traced as seen through the microscope, the position of the real right and left sides is reversed.]

Stages C and D. The letter *H* is placed in the archenteric cavity. (Obj. D, oc. 2.)

FIG. 24.—Transverse section through posterior end of the same larva as the preceding. The transverse band of cilia and the point at which the blastopore has closed are cut through. (Obj. C, oc. 2.)

FIG. 25.—Longitudinal median vertical section through same larva as preceding. Constructed from the series of transverse sections, in which those represented in Figs. 23 and 24 occur.

Figs. 26—34 represent some of a series of transverse sections taken through a larva in Stage E. The epiblast is represented diagrammatically. In each case the upper side is the dorsal one. (All, excepting Fig. 31a, were drawn with Obj. D, oc. 2.)

FIG. 26.—Section through præoral lobe, showing the amœboid cells forming the wall of the mesoblastic sac.

FIG. 27.—Section through the foramen, by which the anterior body cavity opens into the archenteron. The space lying ventral to the hypoblast is the slight ventral backward prolongation of the anterior body cavity, which would disappear in the next few sections.

FIG. 28.—Through the ends of the posterior horns of the anterior body cavity, whose cavities are not prolonged to this point. In this section the rudiment of the nervous system is shown.

FIG. 29.—Section between the anterior and middle mesoblastic regions.

FIG. 30.—Section through the junction of the second body cavities and the archenteric cavity.

FIG. 31.—Section through the middle of the second body cavities.

FIG. 31a.—Part of a section similar to the preceding. It represents the minute structure of the epiblast and hypoblast, and of the mesoblastic cells lining the second body cavity, which are here still connected with the hypoblast. (Obj. F, oc. 2.)

FIG. 32.—Section through the region between the second and third mesoblastic tracts.

FIG. 33.—Section through the anterior region of the third mesoblastic tracts.

FIG. 34.—Section taken rather behind the preceding, traversing the foramina by which the posterior body cavities open to the archenteron.

FIG. 35.—A longitudinal median section of a larva between Stages D and E, showing the opening of the anterior body cavity into the archenteron. (Obj. C, oc. 2.)

FIG. 36.—Longitudinal nearly median section, almost in the vertical plane, through a larva between Stages E and F. (Obj. C C, oc. 2.) The anterior body cavity is now seen to be closed.

FIG. 37.—Transverse section through the mouth of a larva between Stages F and G. (Obj. D, oc. 2.)

FIG. 38.—Transverse section through the anterior end of the collar of a larva between Stages F and G (slightly older than the preceding). (Obj. D, oc. 2). The nervous system is shown still continuous with the skin in this region.

FIG. 39.—Transverse section through the same larva as Fig. 38, taken somewhat behind the section there represented. (Obj. D, oc. 2.) The nervous system is here detached from the skin, to become continuous with it again posteriorly.

FIG. 40.—A diagram representing the arrangement of the parts as seen in a median horizontal longitudinal section through a larva at Stage E. In this, as in Fig. 41, the epiblast is of the medium shade, the hypoblast dark, and the mesoblast light.

FIG. 41.—Diagram of a longitudinal vertical section through a larva between Stages F and G. The letter *D* indicates the dorsal side; shading as in Fig. 40. The anal perforation is here represented in order to show its eventual position. It would not, however, be found until Stage G is reached. The relations of the closed præoral body cavity and of the nervous system to other structures are here shown.

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**On Recent Researches into the Origin and  
Morphology of Chlorophyll Corpuscles  
and Allied Bodies.**

By

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With Plate XXII.

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THE readers of the 'Quarterly Journal of Microscopical Science' will already be acquainted with the earlier researches of A. F. W. Schimper, "On the Development of Starch-grains," a translation of his paper, published under the above heading in the 'Botanische Zeitung' (1880), having appeared in vol. xxi (1881) of this Journal.

Not only did his observations detailed in that paper confirm those of Sachs and Naegeli on the formation of starch-grains in the chlorophyll corpuscles of green parts of plants, but they led him also to a clearer knowledge of the mode of formation of starch-grains in parts of plants which are not green. He found "that starch-grains, which are there in process of development, are not surrounded by ordinary protoplasm, but that they are contained in, or attached to, peculiar highly refractive corpuscles; these are usually spherical or spindle-shaped," and are present before the starch-grains. Their reactions showed that they consist of albuminoid substances, and he concluded that their function is the conversion into starch of the assimilated substances which have been conveyed from other parts of the plant. On this ground he gave them the

name of "starch-forming corpuscles" (Stärkebildner). It is true that these bodies had already been recognised by other writers,<sup>1</sup> and even clearly represented in drawings; further, that Crüger had specially pointed out his "Uebergangsubstanz" as the substance which is in the very process of being converted into starch, and discussed the question of its containing nitrogen; Naegeli<sup>2</sup> and Trécul, on the other hand, scouted this idea. In their hands the structural question, whether a specialised mass of protoplasm is or is not present in connection with growing starch-grains, became so involved with, and absorbed in what was to them the larger question, viz. whether the starch-grains grow by apposition or by intussusception, that they failed to apprehend that fundamental structural point recently brought out by Schimper, which will now be looked upon as the key to their larger question. No doubt the imperfection of microscopical methods had much to do with the long dormancy of this important subject, and it may be safely concluded that improvements in methods have given the impulse to those recent researches, which it will be the object of this essay to discuss.

First among the writers of this period of revival comes Schimper, and no doubt to him belongs the merit of again breaking up the old ground.<sup>3</sup> In the same year as his first paper was published, the Inaugural Dissertation of Dehnecke appeared, dealing with the subject of "non-assimilating chlorophyll bodies." His results harmonise on the whole with those of Schimper, and consequently do not agree with the description given by Molisch and Haberlandt ('Bot. Zeit.,' 1877, No. 23). According to these authors the assimilating chlorophyll bodies arise, in starchy cotyledons, by the surrounding of starch-grains by chlorophyll substance; this

<sup>1</sup> Naegeli, 'Zeitsch. f. wiss. Bot.,' 1845, Heft i, p. 149; iii, p. 109. Crüger, 'Bot. Zeit.,' 1854, p. 41, comp. figs. 12, 13, 14. Trécul, "Des formations vésiculaires (1858)," 'Ann. Sci. Nat.,' 4<sup>ème</sup> série, tome x, p. 234.

<sup>2</sup> Naegeli, 'Stärkekörner,' p. 220.

<sup>3</sup> N.B.—His paper above-mentioned was forwarded from America, and was received by the editors of the 'Bot. Zeit.,' July 27, 1880.

Dehnecke does not find to be correct; he rather concludes that the "etioline bodies" are formed freely in the protoplasm, and become green later. In his further conclusion, that assimilating and non-assimilating chlorophyll bodies are not different, and that in many cases the same bodies may have successively the function of storing starch and of assimilation, and vice versa, his results coincide with those of Schimper.

Up to this point, though the formation of the chlorophyll bodies had been touched upon by various writers, still no clear idea had been obtained of their ultimate origin; and though it was well known and taught in all the text-books that chlorophyll grains could multiply by division, still an origin by free formation was ascribed to them, and it was compared with the process of "free cell formation." The way for a new view of the ultimate origin of chlorophyll bodies and allied structures was prepared by the results of the investigation of the nucleus of the cell by various observers, but especially by Strasburger. This author distinctly laid down the principle<sup>1</sup> that there is no free nuclear formation in embryo sacs, which were formerly understood to supply the best examples of this process. He further widened his generalisation so as to include all tissues, and a translation of his own words will best convey his meaning:<sup>2</sup>—"We are unable any longer to quote examples of free nuclear formation in the vegetable kingdom. . . . In all known cases in the vegetable kingdom cell-nuclei which make their appearance are to be regarded as derivations of pre-existing nuclei. The new nuclei result from a division of older ones."<sup>3</sup>

This same character was found by Schimper<sup>4</sup> to apply also to bodies which we are now considering. His observations led

<sup>1</sup> 'Bot. Zeit.,' 1879, p. 265.

<sup>2</sup> 'Zellbildung und Zelltheilung,' 3rd ed., 1880, p. 321.

<sup>3</sup> Observations recently made do not tally with this statement. Cf. K. Prohaska, "Der Embryo-sac u. d. Endosperm-bildung in der Gattung Daphne," 'Bot. Zeit.,' 1883, No. 52.

<sup>4</sup> 'Bot. Centralbl.,' 1882, No. 44, which is a preliminary abstract of the paper published, 'Bot. Zeit.,' 1883, Nos. 7—10.

him to conclude that chlorophyll bodies, starch-forming corpuscles, and colouring bodies such as are found in the cells of flowers and fruits, are closely related bodies; and further, that they all have a common origin. As in the case of the nuclei of cells, they are the results of division of colourless spheres, which coincide in their material characters with the starch-forming corpuscles previously described by him. These spheres may be found in all growing points; in some few cases chlorophyll may be found, even in the tissue of the growing point. He states also that in the growing points themselves these structures do not originate by differentiation from the cell-plasma, but they are the products of the division of similar starch-forming corpuscles or chlorophyll bodies, which may be found in the embryo while still young (figs. 1 and 4). It is probable that these structures are never formed free in the protoplasm, but, like the nuclei of cells, are continued by division from one generation to another.

So great an advance on our former views of these bodies and of their relations one to another made it necessary to adopt a new terminology, and Schimper has proposed the following, which appears to answer the requirements of the situation. He applies to the whole series of these closely related bodies the general term *Plastids*,<sup>1</sup> and divides them into three groups, which are named according to their most prominent characteristics, viz. according to their colour, as follows:—The starch-forming corpuscles are termed *Leukoplastids*, since they have no definite colour, but are a yellowish white; the chlorophyll granules are called *Chloroplastids*; and to the colouring bodies of flowers and fruits the name *Chromoplastids* is applied. Of these several categories the *Chloroplastids* are always originally derived from *Leukoplastids*, and the *Chromoplastids* from *Leuko-* or *Chloroplastids*. Thus the products of the *Leukoplastids* which are found in *Meristems* have a different fate, according to the organs

<sup>1</sup> It may be objected that this term has already been applied by Haeckel ('*Generelle Morphologie*,' Bd. i, p. 269) in another sense, but the term has never obtained a hold in botany in the sense in which it was used by Haeckel.



or tissues in which they are included, or, in other words, they are capable of a varying metamorphosis. Some of them remain as Leukoplastids, and serve for the formation of starch at the expense of substances already assimilated; or they become Chloroplastids in green-coloured parts; or finally, in flowers or fruits, they assume various colours, and appear as Chromoplastids. Further, as above implied, one and the same plastid appears to be capable of various metamorphoses; thus, Leukoplastids may develop as chlorophyll grains, and subsequently lose their green colouring matter, and assume the character of Chromoplastids. Lastly, it has been observed in the fruit of *Symphoricarpus racemosus* that Chloroplastids may be again transformed into Leukoplastids, and the same is the case in many embryos.

Some plastids have an active life; they assimilate, or produce starch at the expense of materials already assimilated, they form pigments, multiply by division, &c. But there are also plastids which have temporarily or permanently little or no vital function. This is the case with many Leukoplastids, especially those found in the epidermis of most plants, and also the colouring bodies of flowers and fruits. With this passive character the plastids assume more or less crystalline forms and are also doubly refractive. (Figs. 8—14). The active plastids are, however, always round in the higher plants. Schimper further describes how the originally round active plastid becomes transformed, rapidly or slowly in various cases, into the angular, crystal-like plastid (compare Figs.), and how conversely the crystal-like plastid may again resume the spherical form when it again begins to exercise active vital functions. He finds himself convinced that we have here to do with a true process of crystallisation, and that the angular plastids not only have an external similarity to crystals, but actually are true crystals.

In the main points these results are supported by the independent observations of Prof. Strasburger, and A. Meyer.

<sup>1</sup> 'Bot. Centralbl.,' 1882, No. 48, "Ueber Chlorophyllkörner, Stärkebildner, und Farbkörper," 'Das Chlorophyllkorn,' Leipzig, July, 1883, Felix.

The latter observer,<sup>1</sup> who appears to have suffered some delay in the publication of his results, confined his attention to the study of the plastids in the Angiosperms; and, having investigated for the most part the same questions as Schimper, he arrives at conclusions which coincide nearly enough with those already mentioned in this paper to show that the observations of both investigators are worthy of credit in all the most important points.

Unfortunately these two authors, working and writing at different institutions at the same time, have adopted different terms for the same structures, and it may assist those who will subsequently read the papers on this subject, if a table of synonyms be given, which may serve as a key to connect the terms used by these authors one with another and with the older nomenclature.

|                                                                                                                | Older Terminology. | Schimper.      | Meyer.       |
|----------------------------------------------------------------------------------------------------------------|--------------------|----------------|--------------|
| General term. } . . . . .                                                                                      |                    | Plastid.       | Trophoplast. |
| Special terms. { 1. Includes starch-forming corpuscles, and also colourless plastids which do not form starch. |                    | Leukoplastid.  | Anaplast.    |
| 2. Chlorophyll granule.                                                                                        |                    | Chloroplastid. | Autoplast.   |
| 3. Colouring granules (?).                                                                                     |                    | Chromoplastid. | Chromoplast. |

Priority is a strong argument in favour of the nomenclature of Schimper, and further, the terms which he employs explain themselves, which cannot be said for those of Meyer. For these reasons I have decided to retain the terms proposed by Schimper.

It will be unnecessary here to enter into detail on the results of Meyer's observations. It will answer the present purpose better to note only the more important points in which he differs from Schimper, or where he has covered ground which was not touched by Schimper.

Meyer enters more into detail as to the chemical composition and minute structure of the plastids than Schimper; for details readers must be referred to the original work. It is,

however, as the result of this part of his study that he arrived at conclusions which diverge from those of Schimper. These may be shortly stated as follows. The Trophoplasts (plastids) have always a skeleton which is resistant to ordinary dissolving media; they also contain substances easily extracted by various dissolving media, which are often embedded in the skeleton as larger or smaller granules: of the latter the best known are chlorophyll and xanthophyll. When the Trophoplast (plastid) develops as a typical Autoplast (Chloroplastid), the skeleton is bulky and includes large quantities of chlorophyll; when it develops as a typical Chromoplast, only a meagre skeleton is formed, and this is again partially absorbed long before the death of the cell; while the xanthophyll, which is chemically allied to chlorophyll, and is the yellow substance of Chromoplasts in flowers and fruits, is present in large quantities.

The latter substance is insoluble in water, and may be obtained macrochemically as yellow crystals, similar to those which are found in flowers and fruits. Not only may all three derivatives of Trophoplasts include starch-grains, but also Autoplasts (Chloroplastids) and Anaplasts (Leukoplastids) may contain crystalloids.

Thus the point at issue between Schimper and Meyer is this: the former holds that the crystalline form of non-active plastids is that which would be assumed by the substance which forms the proteid basis or skeleton of the plastid, while the colouring matter is carried mechanically with the crystallising proteid, or is extruded from it, and remains attached to the surface of the crystal. Meyer, on the other hand, maintains that the crystalline form is that which would be assumed by the colouring matter, or by a crystalloid, independently of the basis or skeleton, which, in cases where crystallisation occurs, is previously reduced in bulk.

The controversy on this and allied points is still continued,<sup>1</sup> but the details of it are hardly suitable to a paper like the present, and readers must be referred to the original publica-

<sup>1</sup> Meyer, 'Bot. Zeit.,' 1883, Nos. 30—32. Schimper, 'Bot. Zeit.,' 1883, No. 49.

tions. It is plain that whichever view be correct, the effect upon our conception of the morphology of the cell and of its contents would not be greatly affected: it is a question rather of micro-chemistry and physics than of morphology.

Thus far attention has been paid chiefly to the plastids occurring in the tissues of the higher plants. Great advances, however, have also been recently made in our knowledge of the corresponding bodies in the lower organisms, especially through the labours of Prof. Schmitz.<sup>1</sup>

He applies the term chromatophore to all those definite organs of the protoplasm which are coloured green, red, or brown. All these bodies are morphologically analogous, and only differ in their colour.

At the outset the Cyanophyceæ (or Phycochromaceæ) are excluded from consideration, on the ground that they, unlike the other three divisions of Algæ, have no clearly defined chromatophores, or nuclei. He prefers to divide the Thallophyta (excluding Myxomycetes) into three sections.

1. Algæ (including Chlorophyceæ, Phæophyceæ, and Rhodophyceæ).

2. Fungi.

3. Schizophyta (including Schizomycetes and Phycochromaceæ).

In the Algæ as above defined the chromatophores are always definitely limited bodies, and the colouring matter is never equally distributed throughout the protoplasm of the cell. This statement is in direct opposition to the prevalent ideas; it is usual to describe many green Algæ as having uniformly green coloured protoplasm, in which may or may not be embedded chromatophores of definite form. Professor Schmitz, however, asserts that, in the very large number of Algæ which he has investigated, all have chromatophores of definite form, and he confidently extends that generalisation to all Algæ. It should be noted that Professor Schmitz recommends living cells only as giving safe results; unhealthy material, or such

<sup>1</sup> 'Die Chromatophoren der Algen,' Fr. Schmitz, 1882. Bonn, Cohen and Sohn.



as has been killed by glycerine, or other reagents, often gives the appearance of a uniformly coloured protoplasm, and this has no doubt given rise to the prevalent idea of an absence of definite chlorophyll bodies in certain cases.

Such chromatophores may be present singly, or in larger numbers in the cell; their form is very various, but their general form, and their number in the individual cell, are constant for any given species.

The simplest form is that of a small flat disc, of round or irregularly angular outline, as in the Characeæ, Siphonæ (fig. 15), and various other Chlorophyceæ, &c., and the great majority of the Phæophyceæ, also in very many Rhodophyceæ. Closely connected with this simplest form of chromatophore is that with a more or less lobed margin, while the most varied transitional forms lead to large flat discs with various development of their contour. The latter form is most common among the Chlorophyceæ, and less frequently found among the red and brown Algæ, narrow and band-like chromatophores being more common in these groups. A further deviation from this discoid form of the chromatophores is shown in many Chlamydomonads, Volvocinæ, Palmellaceæ, &c., in which in accordance with the spherical form of the whole cell of these plants, the chromatophore is moulded into a hollow spherical form, while it is strongly thickened in the middle, so that the whole assumes the shape of a watch-glass; sometimes, however, this central thickening is so strong that the chromatophore has an almost spherical form, with only a slight flat hollowing at one side, as in *Pleurococcus*, *Palmella*, *Volvox*, &c.

Far more complicated forms result from the formation of narrow bands not at the margin, but on the surface of the discoid chromatophore. Such processes are not uncommonly found as median weals on the inner surface of the chlorophyll bands of *Spirogyra*, or the plates of *Mesocarpus*, &c. But where several such weals traverse the chlorophore longitudinally, and on both sides of it, a form is attained such as in *Micrasterias*, while a further development of the same sort is

found in *Closterium*. In the various complicated forms which are thus produced there may still be recognised a rod like central portion, which, however, is sometimes so short as to be almost spherical. The stellate form which is thus attained has long been known as characteristic of *Zygnema* (fig. 16).

Though the chromatophores are so variable in form, their arrangement and distribution within the individual cell are usually constant, and only in few *Algæ* has it been found that they move from place to place (e.g. in many *Siphonææ*). An analogous regularity of arrangement holds also for the cell nuclei; and the conclusion forces itself upon the observer that there is often a certain mutual dependence between the two organs in the cell, inasmuch as the position of the nucleus is clearly determined by the arrangement of the chromatophores. It appears that the chromatophores are always completely enclosed by colourless protoplasm, though it is often difficult to be certain of this point.

The minute structure of the chromatophores shows great uniformity: when in the living state, whether they be green or red or brown, they usually appear under high powers as quite homogeneous, and without any special internal structure. Still their substance is never quite transparent, and sometimes has a finely dotted appearance, as though there were a very finely porous, or reticulated structure. This appearance is heightened after killing by reagents. Professor Schmitz finds the chromatophores to consist of a ground substance allied to protoplasm, and colouring matters diffused through it; and he gives as his opinion that the colouring matter does not fill the cavities of the network, but that the reticulated ground-substance is itself permeated by the colouring matter.

Many chromatophores are without further peculiarities of internal structure: e. g. most *Floridææ*, many *Phæophyceæ*, all *Characeæ*, and some *Chlorophyceæ*, *Vaucheria*, *Codium*, *Botrydium*. In other cases including the large majority of green *Algæ*, one or several, usually spherical bodies are embedded in the chromatophore, just as nucleoli in the body of the nucleus (fig. 15). These are termed *Pyrenoids* (*πυρήν*—stone of a fruit). In

their simplest forms, as in marine Diatoms, and some Florideæ, these pyrenoids appear as small, round, highly refractive granules, of which one is found in each disc-like chromatophore. The structure is often complicated by the aggregation of numerous starch-granules round the pyrenoid. These aggregations, together with the pyrenoids contained in them, have long been known among green Algæ under the terms "chlorophyll vesicles," "amylum bodies"<sup>1</sup> (*Amylumheerden*, *Amylumkerne*, *Amylumkugeln*) (figs. 16 and 17). The starch grains surrounding the pyrenoid may remain distinct, or they may fuse laterally so as to form a continuous mass. The number of pyrenoids, their arrangement and distribution in the chromatophores, sometimes show great regularity; in other cases, and especially in large discoid chromatophores, there seems to be no constancy in these respects.

The pyrenoids themselves are colourless, spherical bodies, consisting of a dense, highly refractive substance. This is a point which it is difficult to observe; when viewed under a low power it appears as though the pyrenoids were more deeply coloured than the rest of the chromatophore. But observation with high powers shows clearly that they are colourless, dense, refractive bodies embedded in the coloured mass of the chromatophore; and this being the case, they would naturally appear of a darker colour than the surrounding mass of the chromatophore when viewed by transmitted light, and under a low power. These colourless bodies appear to be homogeneous when examined under high powers and in the living state. Here again a reticulate structure appears under the action of reagents, but Professor Schmitz is of opinion that this structure does not pre-exist. An investigation of the behaviour of the pyrenoids before various reagents leads him to the conclusion, that the substance of which they are composed is, in its chemical nature, nearly allied to that of the chromatin-bodies (*nucleoli*) of the cell-nucleus, though at present it is impossible to state definitely what the special nature of the substance is.

<sup>1</sup> Cf. Sachs, 'Text-Book,' 2nd Engl. ed., p. 258.

It has been above stated that the pyrenoids are in many cases, and especially in the green Algæ, surrounded by aggregations of starch-grains, which sometimes fuse together so as to form a starchy sheath (*Amylumheerd*). These starch-grains and sheaths lie completely outside the pyrenoid, and are embedded in the substance of the chromatophore. This shows that the pyrenoid does not directly take part in the formation of the starch-grains, though from the constancy of the position of the latter, the idea may well be entertained that the pyrenoids give rise to a soluble substance, which is transformed into starch in the surrounding substance of the chromatophore.

The pyrenoids are multiplied in two ways: either by division into two, or by a new formation.

The division is naturally most simple in the case of the pyrenoids which have no starch-sheath. As in *Bryopsis plumosa* (fig. 15), where the stages of the process are simply elongation, median constriction, and ultimate complete separation of the two terminal parts. A new formation of these simpler forms of chromatophore has also been observed with certainty in some cases, though the absolute proof of such a process is a matter of considerable difficulty. They appear first as very small spherical bodies of substance similar to that of the mature pyrenoid, and increase gradually till the normal size is attained.

In the case of the more complicated pyrenoids with starchy covering, the division is most readily followed in those examples where there is but one in each cell, which divides also into two parts on the division of the cell (fig. 17).

Here the division of the pyrenoid proceeds as in the above simpler case; the sheath of starch-grains at first remains unaltered, but later, with or without a slight previous constriction, the sheath is completed round each of the new pyrenoids by the formation of two layers of fresh starch-grains parallel to the plane of division.

As might be expected, these processes are often complicated and varied in different cases; and as an example may be taken



the genus *Zygnema* in which the pyrenoid is always surrounded by a sheath composed of numerous starch-grains; but here the division of the sheath may not run parallel with that of the pyrenoid, the result being that one sheath may enclose two or more pyrenoids.

A new formation of pyrenoids has been directly observed by Professor Schmitz in the simpler case above mentioned, though absolute proof of a question like this is difficult. And apparently a similar process goes on in the case of those of the more complicated type. In general, however, the increase by division of pyrenoids is by far the most common.

Returning now to the study of the chromatophore at large, it is found that these bodies also multiply by division, and several varieties of the mode of division are described, the most important being division into two, while sometimes a division into more than two may be observed. Further, in the first case the division may be into equal or into unequal parts. In those cases where there is a single pyrenoid, the division is often closely connected in point of time with that of the whole chromatophore (fig. 15); and the comparison might be drawn between this and the behaviour of the nucleus in ordinary cell division. The same argument, however, may be brought to oppose a view of a definite causal dependence of these processes one on another, as has been already advanced in the case of cell division, and division of nuclei; viz. that as the division of polynucleated cells is independent in time of the division of the nuclei, so also is the division of chromatophores with many pyrenoids independent of their division.

Professor Schmitz states boldly that there is never any other increase in number of chromatophores in the cells except by the process of division. Here he is at issue with the statements of many observers. He draws his conclusions, however, from the careful observation of many forms, during different periods of their life. He compares this character of the chromatophore with that of the nucleus above alluded to. A further point in common between these bodies is that of copulation or fusion; this has been observed in the

chromatophores of conjugating cells of *Spirogyra*, and also in other examples.

The disappearance of chromatophores by resorption has been seen in various examples, often in cells which are destined exclusively for a definite function. This process of resorption is accompanied, or rather preceded by a disappearance of the pyrenoids also; but the disappearance of a pyrenoid has never been observed in active vegetative cells. This fact is strong evidence that the pyrenoid is not a lifeless body included in the chromatophore, but rather an actively living part of it; the attempt on the part of Meyer ('*Bot. Zeit.*,' 1883, p. 493) to show that the pyrenoids described by Schmitz are really crystalloids fails in a very essential point; since he admits (p. 494) that he has never seen the pyrenoid disappear entirely in specimens of *Spirogyra*, which he had starved.

For further details, and especially on the bodies contained in chromatophores, readers must be referred to the original paper.

In conclusion, a few general remarks upon the bearing of the remarkable discoveries above described may not be out of place. We have seen how three separate observers, working separately,<sup>1</sup> and almost simultaneously, have arrived at very similar results; it is rarely that such unanimity is attained, and we must regard the fact as establishing those main points in which their evidence agrees, upon an unusually firm basis. I refer especially to the conclusion that, as far as experience goes as yet, plastids are not formed free in the protoplasm, but are the products of division of pre-existing plastids. It must be allowed, however, that the proposition cannot be at present stated without the limitation given above. It still remains to be shown among vascular plants (1) how the plastids are transmitted, if indeed they are transmitted, from the parent plant to the oosphere; and (2) what part they take in the process of fertilisation. Another point of great interest will be to ascertain whether the distribution of plastids in one form or another be coextensive with that of nuclei; or in other

<sup>1</sup> Prof. Schmitz made at least the most important of his observations at Naples, not at Bonn.

words, whether such bodies are to be found in the cells of fungi; and in connection with this it may be noted that plastids have been observed in colourless Phanerogamic plants.

Until these points are decided we shall be unable to draw a complete comparison between the plastid and the cell nucleus. We have, however, already at hand sufficient knowledge of the subject to allow of some very interesting comparisons being drawn between these two categories. At the conclusion of his paper, Professor Schmitz offers some remarks of the greatest interest on this subject, which bring out clearly in how many points the cell nucleus resembles the chromatophore. Both are formed, at all events in the very large majority of cases, if not exclusively, by division of pre-existing similar bodies; both consist of a ground-substance with a finely reticulate structure, which resembles the protoplasm of the cell; this in each case encloses bodies (one or more) with a definite outline, consisting of a substance giving similar reactions in either case; these included bodies behave generally, if not exactly, in a similar manner during the division of the whole; in both cases coalescence may occur. On the other hand, there are points in which the chromatophores and nuclei differ; thus the peculiar phenomena accompanying division, which are so characteristic in nuclei, are not found in chromatophores. On the above and other grounds, Professor Schmitz suggests that the nuclei and chromatophores may be placed side by side as two series, which have been derived from a common starting point, and have undergone individual modification to subserve different physiological purposes. This being the conclusion drawn from the study of the lower green-coloured organisms, the extension of it may be allowed also to the higher plants, the chief difference being that here the chromatophore has undergone further specialisation, as shown in the plastids of the different categories above described.

It remains to consider the relation of these included bodies to the protoplasm which surrounds them. A new and startling view is hinted at by Schimper in a footnote to his paper ('*Bot. Zeitg.*,' 1883, p. 112). He suggests that if it be defini-

tively proved that the plastids are not newly formed in the oospheres, their relation to the organism would to a certain extent remind one of a Symbiosis; and in support of the possible view that the green plants may owe their origin to a uniting of a colourless organism with one uniformly coloured with chlorophyll, he quotes certain observations of Reinke. This observer states that chlorophyll grains from a decomposing cucumber, which were surrounded by filaments of *Pleospora* in dead cells, continued their growth, and divided.

From what has been above stated as to the close similarity between nuclei and plastids, especially among the lower plants, it seems probable that a similar view, if entertained at all, ought to be extended to the nuclei; in which case the protoplasmic basis of the cell would be all that would remain as representing the protoplasmic body of the host plant. Such a view, however, can hardly be rationally entertained on the ground of the isolated observation of a single investigator; and until it rests upon a better basis, it may reasonably be dismissed from the mind. This suggestion is, however, of further interest, as it runs parallel with a similar view stoutly upheld with regard to the chlorophyll bodies in certain animals, a subject which was treated by Professor Lankester in the 'Quarterly Journal of Microscopical Science,' for July, 1882.<sup>1</sup>

<sup>1</sup> I may be allowed to briefly point out here that the recent observations on the chlorophyll corpuscles of plants, so ably summarised in the above article by Mr. Bower, confirm the truth of the proposition which I maintained in the paper referred to, viz. that there is no more reason for regarding the chlorophyll corpuscles of *Spongilla* or of *Hydra* as parasites (as Karl Brandt would do) than there is for so regarding the chlorophyll corpuscles in the leaf of a buttercup. The fact which has been adduced by Haman in favour of Brandt's view, viz. that the egg-cell of *Hydra* is "infected" with chlorophyll corpuscles from its parent's tissues, is apparently no exception to what occurs among green plants. There also the chlorophyll corpuscles are handed on from parent to offspring. The parallelism of the structure of the chloroplastids of plants and animals will be obvious to any one who will compare with Mr. Bower's essay my account of the chloroplastids of *Spongilla* and *Hydra*, or that by Miss Sallitt in the present number of the Journal relating to the chloroplastids of *Stentor*, *Paramecium*, and *Euglena*.

The fact that chlorophyll does not occur even in unicellular *Algae* in a



Finally, the idea naturally suggests itself that there may be some relation between the plastids and those small bodies in the protoplasm which were described by Professor Strasburger under the name of "microsomata," in his work on the structure and growth of cell walls; and there are among his figures some which lend support to the idea, while the reactions of the microsomata do not differ essentially from those of certain plastids. That there should be any uncertainty on this question shows us two things; first, that the difficulties surrounding the study of the origin and relations of those bodies which are included in the protoplasm must be very great; and secondly, that though remarkable advances have been made in recent years, and have modified the current opinions both of the morphology and the physiology of the bodies included in the protoplasm, there are many essential and interesting points still remaining very obscure; these we may confidently expect to see cleared up, provided advances are as rapid in the future as they have been in the immediate past.

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## DESCRIPTION OF PLATE XXII,

Illustrating Mr. F. O. Bower's paper "On Recent Researches into the Origin and Morphology of Chlorophyll Corpuscles and Allied Bodies."

Figs. 1—14 are taken from Dr. A. F. W. Schimper's memoir, 'Bot. Zeit.,' 1883, Nos. 7—10.

Figs. 15—17 are taken from Prof. Schmitz's memoir "Die Chromatophoren der Algen," Bonn, 1882.

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diffused condition in the cell-protoplasm—as it was hitherto believed in some cases to do—renders the observation by Engelmann (confirmed by Miss Sallitt) of the occurrence of chlorophyll in a diffused condition in a green species of *Vorticella*—of even more striking significance than it had when it was adduced to prove that animal protoplasm can form chlorophyll, and is not dependent for it on parasitic corpuscles.—E. RAY LANKESTER.

FIGS. 1 and 2.—(800.) From the stem of *Impatiens parviflora*.

Fig. 1. Cells from the apical meristem.

Fig. 2. Mature chloroplastids. *a*, from the cortex; *b*, from the epidermis.

FIGS. 3—7.—(800.) From the stem of *Tradescantia albiflora*.

Fig. 3. Cells of the apical meristem.

Figs. 4—6. Gradual increase of the plastids. (Picric acid—Hæm: preparations.)

Fig. 7. Mature chloroplastids.

FIGS. 8—11.—(800.) From the epidermis of the flower of *Asphodeline lutea*.

Fig. 8. Young epidermal cell with colourless plastids; it was situated between the guard cells of two stomata.

Fig. 9. Plastids after the formation of starch.

Fig. 10. Chromoplastids during development.

Fig. 11. Mature chromoplastids.

FIGS. 12—14.—(800.) From the epidermis of the flower of *Senecio Ghiesbreghtii*.

Fig. 12. Young cell.

Fig. 13. Older state.

Fig. 14. Cell of the mature bud with chromoplastids.

FIG. 15.—*Bryopsis plumosa*. (Picric acid—Hæm.) Isolated discoid chromatophores dividing. (800.)

FIG. 16.—*Zygnema* sp. (Picric acid—Hæm.) Cell with the two stellate chromatophores in optical longitudinal section. Starchy sheaths of the "chlorophyll vesicles" consisting of numerous very small starch-grains. Pyrenoids contracted. (800.)

FIG. 17.—*Hyalotheca mucosa*. (Picric acid—Hæm.) Isolated cells, or isolated chromatophores, with various stages of division of the "chlorophyll vesicles."

On the Pharynx of an Unknown Holothurian of  
the Family Dendrochirotæ, in which the  
Calcareous Skeleton is remarkably developed.

By

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With Plate XXIII.

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THE specimen of which an account is here given was found amongst a quantity of other material dredged in the Sulu Sea, in from ten to twenty fathoms, by Captain W. Chimmo, R.N., of H.M.S. "Nassau," 1871, and presented by him to the Anatomical Department of the Oxford Museum. The specimen is unfortunately isolated, and no traces can be found of the body of the Holothurian to which it belonged. Its actual length is about  $1\frac{3}{4}$  inches, and because of its large size and the regularity of the arrangement of the calcareous plates with which it is protected, it forms a striking object, which attracts the eye of a naturalist at once. The anterior part of the pharynx itself is complete, with its contained tentacles and calcareous ring, but the retractor muscles are completely torn away, and posteriorly no trace remains of the water vascular ring or its appendages. The membrane which closed the intervals between the rows of radial pharyngealia has also disappeared. The calcareous plates composing the actual ring (annulus) around the anterior region of the pharynx will here be termed annularia; those disposed in rows posteriorly the pharyngealia.

The calcareous ring is composed, as is usual in Dendrochirotæ, of ten skeletal pieces, five radial and five interradial in position.

The radial annularia are roughly triangular in form, with broad bases shaped angularly, so as to fit in between the pharyngealia, which succeed them posteriorly, and elongated apices. These apices are at their tips deeply notched, probably, as in other forms, for the passage of the radial canals of the water vascular system, and the bodies of the plates themselves are perforated, each by an oval aperture, possibly for the exit of nerves, as in *Synapta* and *Echinosoma* but very possibly, also, for the attachment of the retractor muscles of the pharynx. In *Haplodactyla* there are depressions on the radial annularia for these muscles.<sup>1</sup> Or the apertures may represent the bifurcations at the bases of the radial annularia of *Holothuria princeps* and other species, which are believed by Semper to be the homologues of the radial pharyngealia of certain *Dendrochirotae*. The median ventral radial annulare is much smaller than the others; is not perforated nor notched at its apex, and it is partially fused laterally with the adjacent interrarial annularia. The retractor muscle attached to it is very probably smaller than those inserted into the other four radials. The interrarial annularia are longer and broader than the radial, but of a similar triangular shape. They are not perforated or notched. The two ventral interradians are differently formed from the others, being unsymmetrical and pushed inwards towards the ventral middle line, so that the intervals between their anterior processes and that of the ventral radial annulare are much narrower than the remaining corresponding intervals. Opposite these minor ventral intervals internally are attached the two tentacles out of the ten present, which, as in many other *Dendrochirotae*, are much smaller than the rest. Opposite the remaining corresponding intervals are attached the eight larger tentacles. All the tentacles are dendritic (Fig. 2), and placed opposite the intervals between the radii and interradii. They have no ampullae. Behind each of the annularia follows a double row of well-defined calcareous plates (pharyngealia), and the ten double rows of these being closely united, side by side, invest the walls of the pharynx with a continuous calcareous armour. I term these

<sup>1</sup> See Semper, 'Reisen in den Phillipinen. Holothurien,' S. 160.



plates "pharyngealia," to distinguish them from those forming the actual anterior ring "annularia." The radial pharyngealia are of a nearly uniform size and of hexagonal form. They are disposed in each radial interval, with an alternate arrangement so that the lateral prominent angle of each plate on one side of the double row fits into the receding angle formed by the junction of two adjacent plates on the other. In the radial series no accessory ossicles are present. The radial pharyngealia are not quite flat, but somewhat concave on their internal faces, and on their external faces show traces of slight ridge-like thickenings directed radially from their centres towards their angles. They are stout and of considerable thickness, and show microscopically a structure composed of a very close network of short rounded bars of semitransparent calcareous matter firmly fused together. Towards the posterior extremity of the pharynx in the specimen, beyond the points at which the interambulacral pharyngealia cease, the radial pharyngealia become narrowed and elongate antero-posteriorly, and curved towards the cavity of the pharynx so as to form semicanals. These enclose what appear to be the remains of the tentacular canals. Part of this curling may be due to the fact that the membrane connecting the pharyngealia is here torn away.

Whilst the radial pharyngealia show a tendency to be elongated longitudinally, the interrarial are elongated transversely. They are similarly disposed in double rows. Their outer margins are closely applied to those of the radial, and their inner united together much in the same way as those of the radial. In the two lateral interrarial series, however, irregular small ossicles are interposed in the median intervals between the two rows of plates for part of their lengths. The interrarial pharyngealia do not extend so far posteriorly as the radial, and also are not, like the radial, equal in length in all the series. The two ventral series are longest, the single dorsal next in length, and the two lateral shortest, but the extents shown in the figure are not quite certain, owing to the imperfect condition of the specimen. The interrarial pharyngealia are much less solid and compact than the radial, and readily become broken

up into numerous fragments when handled, though the fragments still hang together and the outlines of the plates remain unimpaired. The whole of the plates composing the pharyngeal armour are attached side by side, as in other *Dendrochirota* by membrane, and not articulated to one another.

The present specimen is especially remarkable for its large size. In *Molpadia chilensis* a somewhat similar pharyngeal skeleton was described by Johannes Müller,<sup>1</sup> and a considerable number of *Dendrochirota* of different genera possess a calcareous pharyngeal skeleton composed of separate plates, in addition to the usual simple ring, but in all of these the pharynx is extremely small, scarcely more than one fifth of the size of the present example, or smaller still, and the plates of which the pharyngeal portion of the skeleton is made up are minute microscopic objects, and appear to vary more in form and be arranged with less regularity than is here the case.

The largest pharynx of the kind hitherto described appears to be that of *Thyonidium Schmeltzii* of Dr. Hubert Ludwig,<sup>2</sup> in which it attains a length of  $1\frac{1}{2}$  cm., the entire length of the animal itself being only 6 cm.

The pharyngealia are commonly treated of as merely elongations of the annularia in a posterior direction, accompanied by their subdivision into plates,<sup>3</sup> and some facts concerning the forms of the annularia in various species seem to point in this direction, but in a pharynx such as the present, in which the pharyngealia are so highly differentiated and regularly disposed, it is at least convenient to distinguish clearly between them and the very different annularia which appear to be in themselves adequate homologues of the calcareous ring of most *Holothurians*. Only embryological evidence can satisfactorily determine whether the series of pharyngealia occurring in forms such as the present are really in origin outgrowths of a primitively simple annulus, or, as appears possible, additional independent

<sup>1</sup> J. Müller, 'Ueber den Bau der Echinodermen,' Taf. iv, fig. 1.

<sup>2</sup> H. Ludwig, 'Arbeiten aus dem zoolog. zootom. Institut in Würzburg,' ii Bd., S. 94.

<sup>3</sup> Semper, l. c., p. 159.

skeletal structures developed over an area, that between the annulus and the water vascular ring, which remains devoid of a definite skeleton in the genus *Holothuria* and most other forms; but at all events, the differentiation between the pharyngeal and annular systems of plates has here been extended so far that it is convenient that they should receive distinctive terms.

In the form of the annularia the present pharynx resembles that of *Cucumaria doliolum*,<sup>1</sup> except that in this species the radial annularia are notched below instead of being perforated, and that the interrarial are also notched. It would appear as if the perforation in the radial annularia in the present form were a remnant of a similar notch closed below, or possibly the notch is a degeneration from a perforation. The three ventral median annularia are completely fused in *Cucumaria doliolum*, but closely resemble in form the corresponding plates of the present form. Similar differentiations of the ventral annularia in relation with the reduction in size of the two median ventral tentacles occur in *Ocnus pygmæus*, *Semper*, and elsewhere.

In that the series of interrarial pharyngealia are not extended so far posteriorly as the radial, the present pharynx agrees with nearly all similarly protected, in some of which interrarial pharyngealia may be wanting altogether.

In the general disposition of the pharyngealia it resembles two species of *Cucumaria* and one of *Thyone* figured by *Semper*,<sup>2</sup> and also *Thyone sacellus*, figured by *Selenka*,<sup>3</sup> excepting that in all these instances there are always wide intervals closed only by membrane intervening between the two rows of pharyngealia composing each radial series, whilst in the present instance these plates interlock with one another directly by their edges.

Further, in *Thyone sacellus* the annularia appear as yet indistinctly differentiated from the pharyngealia, being composed of minute separated plates and ossicles. It is difficult to compare closely the arrangement of the minute plates forming

<sup>1</sup> *Selenka*, 'Z. f. W. Z.,' xvii, Taf. xx, fig. 108.

<sup>2</sup> *Semper*, l. c., p. , Taf. xiv, figs. 4—6, Taf. xv, fig. 7.

<sup>3</sup> *Selenka*, l. c., Taf. xx, fig. 115

the pharyngeal skeleton of small forms with that exhibited by the present relatively giant form, in cases in which they are not, as in Semper's magnificent work, figured with especial care, but it appears that the radial pharyngealia are in *Stereoderma Murrayi* (Jeffrey Bell)<sup>1</sup> in direct lateral opposition with one another, as in the present specimen; but here, too, the annularia appear scarcely differentiated. I am much indebted to my friend, Professor Jeffrey Bell, to whom I showed my specimen, for pointing out to me the resemblance to it of the pharynx of this new form of his, and much other valuable information. The spicules in the tentacles of the present form (see plate, fig. 3) are not unlike those of some species of *Thyone*. Professor Bell informs me that he will shortly publish an account of a new *Thyone*, in which the pharynx nearly resembles the present one, excepting of course in size. *Thyonidium japonicum* of Marenzeller<sup>2</sup> is another allied form which has well-developed pharyngealia.

The principal interest of the specimen above described lies, as I before stated, in its large size, and more especially arises from its possible palæontological significance. Had a cylindrical or cup-shaped structure of the size of the present pharynx been found in the fossil condition, similarly composed of symmetrical rows of calcareous plates, it would hardly have been recognised—at all events, had the annularia been imperfect—as *Holothurian*; and it is just possible that the publication of the present account and figure may lead to the recognition of fossil *Holothurian* remains hitherto undetected.

The radial annularia of *Holothurians* being now generally admitted to be homologous with the auriculæ of *Echinoids*, the series of pharyngealia must, had they any homologues in that group, be represented by plates on the pharynx, between the superior extremities of the auriculæ and the water vascular ring; but none such occur, and the series of plates, highly differentiated and regular as they are, appear to be without homologues in other groups of the *Echinodermata* at all, except

<sup>1</sup> Jeffrey Bell, 'Proc. Zool. Soc.,' 1883, Pl. xv, fig. 66.

<sup>2</sup> Marenzeller, 'Verh. zool. botan. Ges. Wien,' 1881, 1882, Taf. v, 9.



in so far as they are represented by the loose spicules which Charles Stewart first showed to be present in the walls of the pharynx of the Echinidæ.

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### EXPLANATION OF PLATE XXIII.

Illustrating Mr. H. N. Moseley's Memoir on "The Pharynx of an unknown Holothurian of the Family Dendrochirotæ," in which the calcareous skeleton is remarkably developed.

FIG. 1.—View of the outside of the pharynx of a Holothurian of the Family Dendrochirotæ opened by an incision along the dorsal middle line, and spread out so that the ventral middle line lies in the centre of the figure. The water vascular ring and digestive tract are torn away from the hinder extremity. Enlarged five diameters. *R.* Radial annulare or component plate of the calcareous ring. *I.* Interradial annulare. *W.* Radial annulare of the ventral middle line smaller than the others, not perforated or forked anteriorly. *V.* One of the two ventral interrarial annularia different in form to the others. *B.* One of the radial series of pharyngealia, or plates covering the pharynx behind the annulus. *C.* Hinder prolongation of another such series enclosing a tentacular canal, and ending in a torn extremity where the water vascular ring was situated. *A.* One of the lateral interrarial pharyngealia, with minute intercalated ossicles. *T.* Slight tentacular pouch, formed by the anterior prolongation of the pharynx.

FIG. 2.—One of the two small ventral tentacles enlarged.

FIG. 3.—Spicules from the tentacles.

## On the Sexuality of the Fungi.

By

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THE fruitfulness and stimulus of the theory of descent have probably been felt in no province of biology with more effect than in the investigation of the more minute forms of plants; and the results obtained from the study of microscopic Fungi have absorbed attention and interest of late years to an extent which, whether commensurate or not with their importance, promises even more in the future than has been attained in the past.

Not only with respect to the economic aspect of a thorough knowledge of Fungi inimical to the animal and vegetable world, but also as regards the real position of these remarkable organisms in nature, it is of the greatest importance that investigations should proceed and multiply. For we have learned in this as in other departments of science, that the results of thorough and accurate knowledge cannot really be foreseen, and that new side lights are thrown on other matters by every acquisition of facts and principles.

Apart from their interests more directly affecting mankind, the Fungi have seemed to present problems of life in some respects simpler than other forms, and have thus in a manner promised a solution of phylogenetic and physiological questions more nearly approaching the ideal of the evolutionist. As research progressed, however, and the methods of observation were improved, experience showed that the study of the Fungi—though yielding results much beyond rather than below what was expected—is attended with unlooked for difficulties. Not

only is the isolation and cultivation of any given fungus an extremely difficult matter, but the following it through all the phases of its life-history brings the observer face to face with problems of quite a special nature.

As time progressed and observations multiplied, it became clear that the Fungi are by no means so simple as they perhaps appeared. Apart from practical difficulties of manipulation, consequent on their minuteness, number, and intermixture with other forms, it soon became evident that special conditions of various kinds affect their development, and that the complete life-cycle of any one fungus—and evidence based on a thorough knowledge of this is alone admissible for the purposes of science—may present various forms of complexity.

Even to-day, notwithstanding the considerable additions to our knowledge derived from the study of developments, and notwithstanding that we possess several comprehensive generalizations as to the curious changes undergone by typical forms in their development, we are far from possessing sufficient knowledge of these matters to enable us to group the Fungi satisfactorily from a phylogenetic point of view. This, however, is a distinct aim of biology, and every addition to knowledge in this direction is to be welcomed.

In the present essay it is proposed to describe some of the more recent and most suggestive observations on Fungi; and especially on their reproductive organs, since it is in these that the most important phenomena (from the phylogenetic point of view) are centred. We shall have occasion to refer to, and in part to trace certain processes connected with their development; and finally to see how far it may be possible to generalise from the facts now known.

In so far as this paper simply recounts observations—for the most part made by others—it cannot claim scientific merit; but if, after condensing and arranging the facts, and stating the condition of our present knowledge of the subject, the attempt to bring this knowledge under a more general statement succeeds, it may be that we have helped to advance matters after all.

If, however, further criticism results in the overthrow of the hypothesis brought forward at the conclusion, we may nevertheless hope that some service is rendered in arranging the facts, and drawing attention to the necessity of employing physiological as well as morphological considerations in the attempt to construct a phylogenetic system of the Fungi. In the last case we may at least succeed in attracting more attention to the direction in which modern research in this region is impelling the more thoughtful biologists, and so call forth confirmatory evidence or criticism of unseen fallacies.

It we neglect a few isolated observations as having led to no general views on the subject, we may regard Pringsheim's discovery of the sexual organs in the *Saprolegniæ* in 1858<sup>1</sup> as the starting point of our knowledge of the sexuality of the Fungi. This observation was made at a time when attention was being drawn particularly to the sexes of the *Algæ* by the researches of Thuret, De Bary, Cohn, Nägeli and others; and the *Saprolegniæ* were then, and for a long time afterwards, regarded as *Algæ*.

Since that time numerous cases of the occurrence of sexual organs have been described among other Fungi, chiefly by the labours of De Bary and the school of cryptogamic morphology practically established by him and his pupils.

With De Bary's brilliant researches on the *Ascomycetes*,<sup>2</sup> and especially the *Erysiphææ*,<sup>3</sup> a point was reached where a definite opinion on the sexuality of the Fungi became accepted; and the conclusions drawn from the study of *Sphærotheca*, *Eurotium*, and *Peziza* led to the view that the fruit-body of a higher fungus results from a process of fertilisation preceding the development of asci, and that Hofmeister's supposition that the asci are sexual organs was to be abandoned.

In 1871 Janczewski<sup>4</sup> described the sexual organs in *Ascobolus furfuraceus*. Other researches by Baranetski, Gil-

<sup>1</sup> Sachs, 'Geschichte der Botanik.'

<sup>2</sup> 'Über die Fruchtentwicklung der Ascomyceten,' 1863.

<sup>3</sup> 'Beiträge zur Morph., &c., der Pilze,' R. iii, 1870.

<sup>4</sup> 'Bot. Zeitg.,' 1871.



kinet, Woronin, Van Tieghem, and Brefeld were considered to support the then generally received opinion that the Fungi, while differing considerably as to their forms and mode of producing spores and fructification, probably all develop their chief reproductive bodies as the consequence of a sexual process.

When in 1874 Stahl demonstrated the sexuality of a Lichen,<sup>1</sup> the matter seemed to be placed beyond doubt; and it was freely admitted that in the cases—now somewhat numerous—where a definite union of sexual organs could not be established, that the failure was largely due to the extraordinary difficulties attending the investigation. In this manner, apparently, it came to be widely believed that in such cases as *Sordaria*,<sup>2</sup> *Penicillium*,<sup>3</sup> *Sphaeria lemaneæ*,<sup>4</sup> and *Chaetomium* the sexual process is essentially the same as that described for the simpler *Erysiphææ*.

All the observers agreed in the main that the Asci are either parts of the Ascogonium, or female sexual organ (*Sphærotheca*, *Podosphæra*), or are developed by budding from it, in each case no doubt after the ascogonium had received something from the male organ (*pollinodium*) attached on its surface. Meanwhile, investigation was not confined to the *Ascomycetes*.

The researches of Brefeld,<sup>5</sup> Van Tieghem,<sup>6</sup> and others<sup>7</sup> demonstrated a simple form of sexual reproduction in the *Mucorini*, now so well known that we need not dwell upon it. It is interesting to note in passing, however, that Ehrenberg had discovered the conjugation of *Syzygites* so long ago as 1820.<sup>8</sup>

<sup>1</sup> 'Bot. Zeitg.,' 1874.

<sup>2</sup> Gilkinet, "Recherches Morphologiques," 'Bull. Acad. r. de Belg.,' ser. 2, 1874. Woronin, 'Beitr. z. Morph. &c.,' R. iii.

<sup>3</sup> Brefeld, 'Schimmelpilze,' ii.

<sup>4</sup> Woronin, 'Beitr. zur Morph. &c.,' R. iii.

<sup>5</sup> 'Schimmelpilze,' H. i.

'Ann des Sc. Nat.,' ser. 6, t. i.

<sup>7</sup> 'De Bary 'Beitr. z. Morph.,' i.

<sup>8</sup> This is the date in Sachs, 'Geschichte der Botanik,' p. 473. De Bary, 'Beitr.,' i, p. 74, gives the date 1829.

We now pass to the discovery of the true nature and sexual organs of the Peronosporæ by De Bary, who, in a series of masterly memoirs,<sup>1</sup> has made this group and its allies a special study. De Bary showed that in certain members of this group an antheridium bores into the oogonium, sending a "fertilising tube" into the oosphere contained therein; the oosphere then becomes an oospore, and capable of germination.

A considerable amount of labour had been devoted to the study of the Saprolegniæ since Pringsheim's first publication, much of it by this investigator himself,<sup>2</sup> and among other remarkable discoveries his observation that, in certain cases, the oospores become normally developed and capable of germination without any male organs being formed at all, is to be noted. Pringsheim himself termed these oospores parthenogenetic.<sup>3</sup> We may pass over the controversy between Cornu<sup>4</sup> and Pringsheim as to certain details in the manner of fecundation of the oospheres of Saprolegniæ. It is sufficient to note that in 1880, or thereabouts, the matter appeared to stand thus: While the typical Saprolegniæ possess oospheres in an oogonium, and antheridia as simple or branched structures which send "fertilising tubes" through the walls of the oogonia as far as the oospheres, which they appeared to fertilise; there are others in which the oospheres develop into fertile oospores without contact of the antheridia.

If we now turn aside from the Fungi referred to in the preceding sketch, we find a vast number of forms comprehended under the Ustilagineæ, Uredineæ (*Æcidiumycetes*), and the larger Basidiomycetes. The parasitic Ustilagineæ have received much attention since Tulasne<sup>5</sup> and De Bary<sup>6</sup> brought them together and led the way to a more scientific knowledge of their nature. Much has been done since, and

<sup>1</sup> 'Ann des Sc. Nat.,' ser. 4, vol. xx, and later, 'Beiträge zur Morph.,' R. ii; ditto, R. iv. 'Bot. Zeit.,' 1881.

<sup>2</sup> 'Jahrb. für wiss. Bot.'

<sup>3</sup> 'Jahrb. für wiss. Bot.,' ix.

<sup>4</sup> 'Ann des Sc. Nat.,' ser. 5, t. xv, &c.

<sup>5</sup> 'Ann. des Sc. Nat.,' ser. 3, t. vii, and ser. 4, t. ii.

<sup>6</sup> 'Die Brandpilze,' 1853.

much opinion has been expressed as to the signification of the cross unions made by the "sporidia" developed from the promycelium of the germinating spores<sup>1</sup> in some cases. We must regard the view as to its supposed sexual character with grave suspicion.

The Uredineæ, apart from their interest as parasites on economic and other plants, have absorbed much attention from the point of view we are concerned with. It was natural to look for sexual organs in them, especially after the successes met with elsewhere. Nevertheless, from Tulasne<sup>2</sup> and De Bary's<sup>3</sup> earlier investigations, more than thirty years ago, down to the present time, no one has succeeded in demonstrating even a trace of any intelligible sexual process or organs. This is the more remarkable since many of the *Æcidiomycetes* produce no less than four forms of reproductive bodies. Moreover, the group has been studied with extraordinary success, and our knowledge of the nature of parasitism and heterococcism is largely if not chiefly due to this success. The best views as to the reproduction of these Fungi held up to 1880 may be fairly stated thus. They form at most two kinds of asexual spores (uredospores and teleutospores) and *æcidia* and *spermogonia*; the latter were regarded as probably the bodies concerned in sexual reproduction, the *spermatia* emitted by the *spermogonia* being the male organs, and the "*æcidium* fruit" probably resulting from a fertilised body equivalent to the *ascogonium* of the *Ascomycetes*. This view was strengthened and supported by Stahl's discovery of the sexual process in Lichens; but no organs like the *ascogonium* or *trichogyne* have yet been discovered in spite of much labour. Finally, we may dismiss the larger *Basidiomycetes* by referring to Brefeld's magnificent research<sup>4</sup> on certain types, and particularly on *Coprinus*.

Brefeld placed beyond all reasonable doubt that the stalked

<sup>1</sup> Tulasne, op. cit.

<sup>2</sup> Op. cit.

<sup>3</sup> Op. cit.

<sup>4</sup> 'Schimmelpilze,' H. iii.

pileus arises from the mycelium, and completes its development without the intervention of any sexual process, or the appearance of any sexual organs; and since no one has succeeded in rendering it probable that sexual organs occur later, we may probably accept Brefeld's view that no sexes exist in the Agarics as we know them, but that they are large aggregations of hyphæ producing asexual spores. Whether we really know the whole life history of any of these forms is a question which cannot be raised with much advantage just now.

It thus appears that while the discoveries of Pringsheim, Tulasne, and De Bary led, on the one hand, to numerous other observations of sexual organs in the Fungi, and seemed to show that a sexual process is nearly universal with them as with other groups of living beings equally complex in organisation; on the other hand, there were numerous cases where room for serious doubts existed—doubts not dispelled by the recognition of the difficulty of the research. As time passed, moreover, the suspicion that certain groups of fungi are really devoid of sexual organs (although analogy would lead us to expect them) increased, and in some cases reached conviction. Of course, we are not referring to the very obscure lower groups—the Schizomycetes, Saccharomycetes, and Myxomycetes, &c.—which we shall leave out of account altogether in this survey.

It is not to be forgotten that much more was known about the physiology of the Fungi by this time, and that the recognition of saprophytic and parasitic forms implied considerable advance in our knowledge of their modes of life, changes of habit, and so forth. The progress made in the study of fermentation, moreover, had its effect on the study of mycology generally; and the progress of biology as a whole—so particularly active during this period—had, in 1880, left its mark on this specialised branch of research.

It is not necessary to enter into all the systems of classification proposed for the Fungi during this period. The well-known grouping of Sachs and Cohn, presented to English readers in the "Text-book" of the former, was admitted to be



in great measure artificial, and those proposed by Van Tieghem<sup>1</sup> and by Winter<sup>2</sup> appear to answer their purpose only temporarily, and certainly need not occupy us here. The same is true for other classifications up to 1880.

It was just prior to this period that the very important, memoir by Brefeld<sup>3</sup> was published, in which he detailed the results of his investigations into the nature of the Basidiomycetes.

By cultivation in nutritive media, Brefeld succeeded in tracing the whole cycle of *Coprinus* from the basidio-spore to the formation of a mycelium and fructification. He shows that the latter arises by a purely vegetative process from the dense mass of interwoven hyphæ (sclerotium) budded off from the mycelium, and that no trace of a sexual process or of the formation of sexual organs can be detected either previously to the development of the sclerotium or afterwards. The pileus with its hymenium are produced simply by a budding off of numerous hyphæ growing up together, either directly from the mycelium, or with the intervention of the sclerotium. Brefeld regards it as certain that these Fungi are entirely without sexual organs.

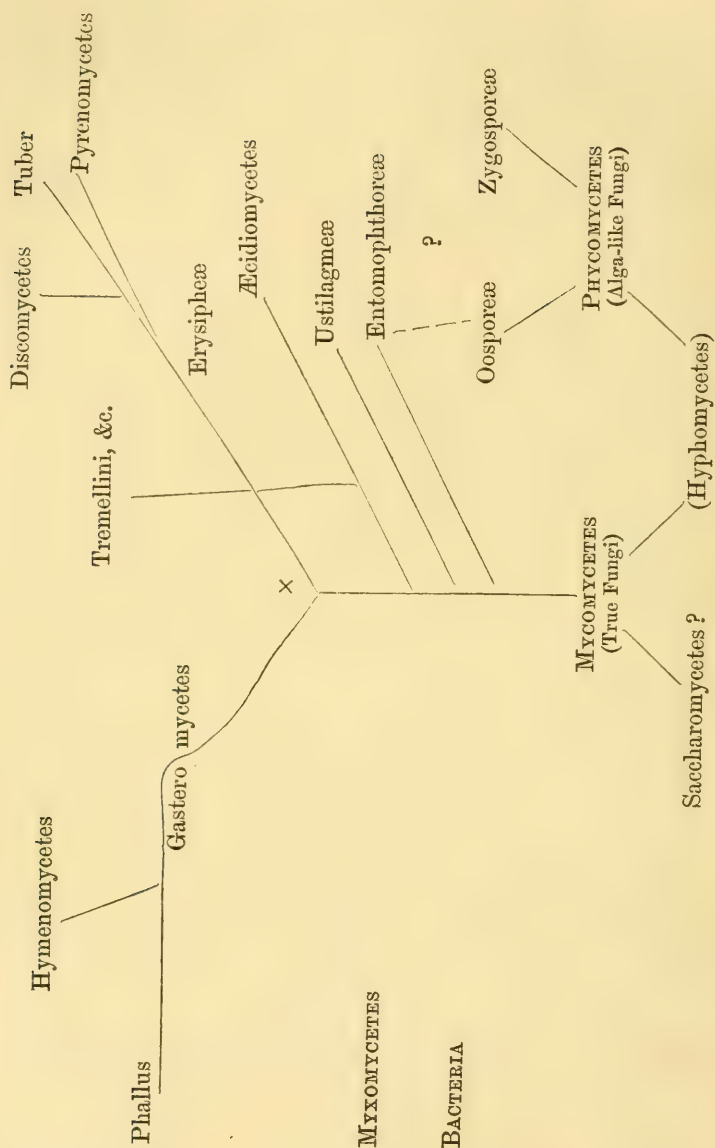
It is impossible to go into the details of this voluminous memoir; but it is important to notice the results embodied in a scheme of a proposed classification of the Fungi which Brefeld tabulates at the end of his valuable paper, since we have here a comprehensive view of the direction in which modern speculations in mycology were tending.

In the accompanying diagram I have slightly condensed Brefeld's scheme, since the original contains details of little importance for our present purpose.

<sup>1</sup> 'Ann. des Sc. Nat.,' ser. 6, t. iv, 1878.

<sup>2</sup> 'Hedwigia,' 1879—see also Rabenhorst's 'Kryptogamen Flora.'

<sup>3</sup> 'Schimmelpilze,' Heft iii, 1877.



There is little need to dwell on this scheme, since its chief interest for us is in its being an intelligible attempt to classify the Fungi from the point of view of the theory of descent. A point of some importance, however, may be referred to, as we shall have occasion to speak of it later. Brefeld indicates the possibility that the Oosporeæ (typified by *Peronosporæ*) may be allied to the "true Fungi" otherwise than by a common descent from some Alga-like ancestor. He also recognises a common origin for the Oosporeæ and the Zygomycetes. In other respects the system is chiefly remarkable for the peculiar views taken of the descent of the two great groups, the typical Basidiomycetes (*Gasteromycetes* and *Hymenomycetes*, &c.) and the Ascomycetes, which he regards as having long ago diverged from a common point, at a time when the ancestral forms commenced to specialise their reproductive organs. While on the one hand asci arose as specialised forms of sporangia—complications resulting from the development of perithecia, &c., being considered unimportant—on the other hand, the sporangia became degraded to conidia, and the Basidiomycetes came to be merely highly developed tufts of conidiophores.

In a later memoir<sup>1</sup> Brefeld insists on regarding the so-called pollinodia of the Ascomycetes as simply tubes for enveloping the ascogenous cell or filament; and it is interesting to note that he quotes *Melanospora* as a case where the non-sexual relation of the ascogenous cell and the filaments which envelope it may be clearly observed. Brefeld also points out that in the Ascomycetes we can trace gradual degradations of the various forms of fructification, with a disappearance of sexuality at the same time. He supposes that all the Fungi arose from an ancestral form containing chlorophyll and possessing sporangia, and that the variations met with are derived by modifications of this sporangium, as already indicated.

It seems unnecessary to criticise these views in detail, since it is obvious that no decision can be arrived at apart from the consideration of numerous facts. It will be noticed, however,

<sup>1</sup> 'Schimmelpilze,' iv, 1881.

that Brefeld's hypothesis assumes that, in addition to purely vegetative modes of multiplication (e.g. the breaking up of filaments, &c.), certain Fungi must have acquired other forms of reproduction than those inherited and specialised—some *Æcidium* mycetes, for instance, with their four kinds of spores or spore-like bodies (æcidiospores, spermatia, uredospores, and teleutospores) must have acquired at least one of these spores.

At this point we may leave this short survey of Brefeld's important work, and turn to the consideration of a memoir<sup>1</sup> published by De Bary about the same time as the last one quoted. In this—probably the most important contribution to mycology yet made—the author describes his observations on the *Peronosporæ* and *Saprolegniæ*; and bases upon these and other previous observations a classification of the Fungi which is in large measure new, and certainly promises to be more fruitful than any yet proposed.

De Bary finds that, passing from the typical *Peronosporæ* (*Pythium*, &c.) to the *Erysiphææ* on the one hand, and to the *Saprolegniæ* on the other, the sexual process is gradually eliminated, and the sexual organs become at first functionless and then disappear altogether. In *Pythium* itself, the antheridium pierces the oogonium wall and fertilises the oosphere by pouring protoplasm into it.<sup>2</sup> In *Phytophthora* and *Peronospora* the process is essentially similar, but the quantity of protoplasm passed over from the antheridium is smaller.

In the *Saprolegniæ*—which differ from *Pythium* and other *Peronosporæ* in forming several oospheres in each oogonium—the fertilising tubes do not open, and no protoplasm can be observed to pass over from the antheridium to the oospheres.<sup>3</sup> Or, in some forms, no antheridia are present at all—a fact already recognised by Pringsheim—and the parthenogenetic spores are nevertheless capable of germinating.

Now if the typical and thoroughly investigated case of

<sup>1</sup> 'Beiträge zur Morph. und Phys. des Pilze, &c.,' R. iv, 1881.

<sup>2</sup> Cf. 'Quart. Journ. Mic. Sc.,' October, 1883.

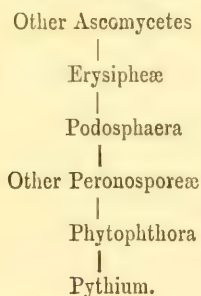
<sup>3</sup> Cf. also 'Quart. Journ. Mic. Sc.,' July, 1883.



*Podosphæra*<sup>1</sup> be compared with the *Peronosporæ*, it is, as De Bary states, evident that the antheridia correspond in both cases; while the "archecarpium" (i.e. the cell which produces the ascus, and to which the antheridium applies itself) of *Podosphæra* is homologous with the oogonium of the *Peronosporæ*. It is a remarkable fact that, as De Bary noticed long ago,<sup>2</sup> the antheridium of *Podosphæra* only applies itself closely to the archecarpium, and does not pierce it; it appears highly probable, moreover, that no passage of substance from one to the other takes place—that the ascus, in fact, arises without a sexual process, though the sexual organs are present.

Now a whole series of forms are known leading us up from *Podosphæra* and the other *Erysiphææ* through the *Pyrenomycetes* and *Discomycetes*, and it is remarkable that (apart from some peculiar forms to be referred to shortly) the best investigations lead us to conclude that while the sexual organs are present, but functionless, in the lowest forms, they disappear entirely in the higher *Ascomycetes*.

These facts may be put shortly in the form of a diagram as annexed, where attention is only paid to the points referred to.

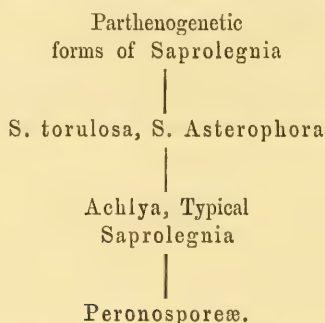


If we now turn to the *Saprolegniæ*, we may note that De Bary finds that, between the typical cases where the antheridia pierce the oogonium wall, but do not empty any proto-

<sup>1</sup> De Bary, 'Beitr. zur Morph. und Phys.,' R. iii.

<sup>2</sup> De Bary, op. cit.

plasm into the oospheres (*Achlya* and some *Saprolegnias*) and the extreme parthogenetic forms of *Saprolegnia* where no antheridia are formed at all, there exist cases where the antheridia apply themselves to the outside of the oogonia, but either form no antheridial tubes at all or only rudimentary ones (*S. torulosa*, *S. asterophora*). These facts may also be diagrammatically represented as follows.



If now the various cases are duly considered, De Bary thinks that we may probably regard the *Peronosporæ* as phylogenetically important in two senses :

1. Their general biology strongly suggests that they are derived from Algal ancestors, possibly not very unlike *Vaucheria* and its allies.

2. That they are the progenitors (or the near relations of progenitors) of a few chief series of true Fungi—on the one hand the main series of *Ascomycetes* and allies ; on the other the *Saprolegniæ* and forms derived from them, and allied to them.

If we now regard these forms more closely, it is not difficult to agree with many of De Bary's conclusions. It will be clear, in fact, that some of them are not new, though they are stated in a much clearer form than by Brefeld and others who have helped to systematise the chief groups already. We will first shortly consider the main subdivisions themselves.

The *Zygomycetes* are regarded as branching off from the *Peronosporæ*. In this group De Bary arranges the Mu-

corini and the Entomophthorææ, basing the conclusion that Entomophthora is a Zygomycete chiefly on Nowakowski's description of the zygospores.<sup>1</sup> It should be remembered that Brefeld considers the resting spores of this genus as arising asexually; but that he, too, indicates the possible alliance of the Entomophthorææ with the Oosporeæ, and therefore, indirectly, with Zygomycetes.

Pythium seems closely allied to the Ancylistææ of Pfitzer,<sup>2</sup> which lead us on to the Chytrideæ, in which we meet with forms which conjugate (and are therefore sexually simpler) as well as purely apogamous genera. It is not improbable that among these latter the asexually produced resting spores are really oogonia, a view already held by Brefeld in other cases.

De Bary raises the point of the possible alliance of these simple parasitic Chytridiaceæ with the lower Algæ (e.g. Protococcaceæ), and decides that it is, on the whole, more probable that they have been derived from the higher Fungi, as indicated, by degeneration.

The Ustilagineæ are next dealt with. The author expresses himself cautiously, but points out that this important and very natural group may be looked upon as a series, beginning with the simpler Entyloma, Tilletia, &c., and rising to more complex forms, such as Sorisporium and Urocystis, on the one hand, and Ustilago on the other.

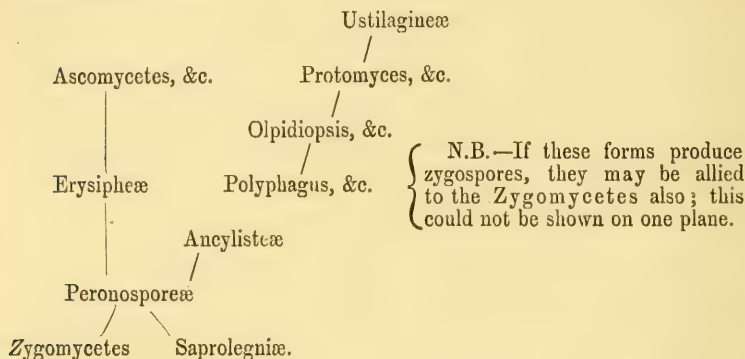
There are many difficult points to consider in classifying the Ustilagineæ. Their asexually produced resting-spores generally form a "promycelium" on germination, from which "sporidia" arise; and, as is known, these "sporidia" commonly become united by cross-unions. But whether this is to be regarded as a "copulation" or not, the sporidia often germinate without it. It should be noted that Woronin<sup>3</sup> points out this significant fact also, and it is all but certain that the so-called "copulation" is not of a sexual nature at all.

<sup>1</sup> 'Bot. Zeitung,' 1877.

<sup>2</sup> 'Monatsber. der Berlin Akad.,' May, 1872.

<sup>3</sup> 'Beitr. zur Morph.,' R. v, 1882.

If the lower Ustilaginæ are allied to the Chytrideæ by means of Entyloma and Protomyces, we seem to have a satisfactory position for the former group. Of course, if this view be accepted, the resting spores of the Ustilaginæ are the homologues of oogonia, which become developed apogamously. The preceding facts may be summarised in the following diagram :



Continuing our survey of De Bary's memoir, we may pass over the opinion respecting the Saccharomyces, and proceed to the part dealing with a much more difficult and important series of forms. As the author showed in 1879,<sup>1</sup> the Tremellini may well be looked upon as derived from Uredineæ and allies; while those Uredineæ which form æcidia resemble the Ascomycetes in so many points of structure and development, that we may regard them as closely allied. The formation of three forms of conidia (uredospores, teleutospores, and sporidia) may be in part due to specialisation; but it must be remembered that the Ascomycetes are also in the habit of forming many and various conidia. It is, however, in the many points of resemblance between the æcidia and perithecia, and the spermatogonia and spermatia of both groups, that the alliance appears most clearly. True, no observer has found a trace of sexual organs in the young æcidia; but the same is certainly true for the perithecia of many Ascomycetes.

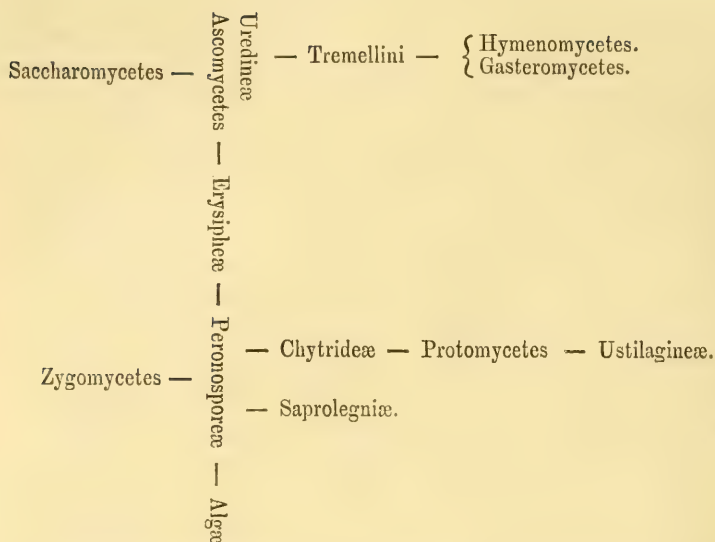
<sup>1</sup> 'Bot. Zeit.,' 1879, p. 825, &c.



In structure, apart from peculiarities in the development of the spores in the two cases—*æcidia* and *perithecia* present many points of agreement, while the *spermogonia* and *spermatia* of both groups are quite alike. It cannot be overlooked, however, that the peculiar development of the *æcidiospores* affects the question of this alliance. De Bary does not even allude to the similarity between these and the “*conidia*” of *Cystopus*; and it is of course obvious that the asexually-produced spores of the latter are rather to be regarded as homologous with one of the conidial forms of the *Æcidiomycetes*; for if the *æcidium* fruit is homologous with the *perithecium* of *Podosphaera*, its further homologies are with the *oogonium*, &c., of *Cystopus*. It has been suggested, however, that a solution of these difficulties should be sought in the direction hinted at here.

De Bary thus considers the *Æcidiomycetes* as a group allied to the *Ascomycetes* genetically, though we have not sufficient knowledge as yet to enable us to place them at any particular spot in the scheme of that series. The *Tremellini* are *Basidiomycetes*, with *basidiospores* so suggestive of the *teleutospores* of *Uredineæ* that De Bary does not hesitate to place them as derived—with considerable reduction and simplification—from those of *Uredineæ* which possess no *æcidia* (e.g. *Chrysomyxa*). This is regarded as no more extraordinary than the peculiar simplification of *phanerogamous* water-plants, &c., or of *Saccharomycetes*, if they are reduced *Ascomycetes*. The *Tremellini* would then lead us to the *Hymenomycetes* and *Gastromycetes*, though it is by no means clear how this came about. We are here plunged into the greatest difficulties, because the development and life-history of these groups are so little known; and we may thus leave the discussion of their phylogeny for the present. It must suffice to add that De Bary believes it possible that the *Tremellini* having arisen by degeneration of *Uredinous* forms, the other *Basidiomycetes* developed anew progressively as forms adapted to special modes of life.

The annexed scheme sums up the whole of the preceding.



The details may be filled in according to what has been said.

On comparing this diagram with that offered by Brefeld, it will be noticed that there is some agreement in general between them; it is chiefly in points of detail that the differences appear. Both the authorities agree as to the serial arrangements (on the whole) of the main groups; but Brefeld, while also placing the Tremellini as derived from Uredineæ, seeks another origin for the other Basidiomycetes. When we notice De Bary's caution in not deriving the Uredineæ from any particular point in the huge ascomycetous series, we may allow that he and Brefeld do not differ much in opinion as to their origin—the latter simply places their origin more definitely lower down in the main series, a fact which would possibly be of significance if we were inclined, after all, to regard the similarities between *Æcidium* and *Cystopus*.

The chief motive in Brefeld's scheme is afforded by his peculiar views on *Pycnidia*, and on the relationships of the *Entomophthorææ*. He regards the point where the main series of higher Fungi developed *pycnidia*, as the point whence

the Basidiomycetes (excepting Tremellini and allies) and Ascomycetes diverge; the former then became specialised as conidium-bearing Fungi, the latter as modified sporangium-bearing forms—i.e. as ascus-bearing Fungi.

The great motive of De Bary's views, as already shown, depends upon the value of the new knowledge obtained of the Peronosporæ, and the meaning of the oogonia (or asconidia) and antheridial filaments respectively.

If, in Brefeld's scheme, we tack the remaining Basidiomycetes on to the Tremellini, &c., and bring his "Oosporeæ" to the base of the main trunk of the phylogenetic tree, slight though not unimportant alterations in detail are necessary to make the two diagrams agree.

If we now turn our attention to the investigations of the last two or three years, it is suggestive that the best results have been won among the Ascomycetes. The influence of De Bary's memoir shows itself repeatedly in these, and they must be regarded as tending on the whole to strengthen his conclusions. No object is to be served by taking the various memoirs in strict chronological order, and I may therefore commence by reviewing an important contribution by Kihlmann.<sup>1</sup> It may first be stated that *Pyronema confluens*, a *Peziza*, is one of the Ascomycetes which has been particularly well studied; and is classical in that De Bary<sup>2</sup> first described the sexual organs in it in 1863, and that Tulasne's celebrated figure<sup>3</sup> of these organs has been so much copied. In 1866 De Bary, having devoted much attention to the development of the fructification of this fungus, wrote as follows concerning the pairs of peculiar organs assumed to be sexual: "Ob und wie sie eine Befruchtung dienen ist eine durchaus unentschiedene Frage."<sup>4</sup>

These sexual organs consist of pairs of swollen branchlets arranged in groups. Each pair consists of a macrocyst and a

<sup>1</sup> 'Acta Soc. Scient. Fenn.,' t. xiii.

<sup>2</sup> 'Über die Fruchtentw. der Ascomyceten.'

<sup>3</sup> 'Ann. des Sc. Nat.,' S. 5, t. vi; cf. Sach's 'Text-book, &c.'

<sup>4</sup> 'Morph. und Phys. der Pilze, &c.,' p. 164.

so-called paracyst. The macrocyst is an ovoid vesicle, filled with protoplasm, &c., and provided with a hook-like tubular prolongation. The paracyst is a club-shaped branchlet, close to the macrocyst; the apex of the paracyst and the hook-like prolongation become united. After this—the “process of fertilization”—branches spring from below, envelope the sexual organs, and form the perithecium, in which the asci arise.

Tulasne<sup>1</sup> described an open communication through the hook-like process, placing the protoplasm of the macrocyst and paracyst in connection. De Bary, much later,<sup>2</sup> speaks with great caution, and considers it undecided that a true sexual process occurs. Fisch<sup>3</sup> doubts whether these organs have anything to do with the formation of the asci. Sachs<sup>4</sup> and Goebel<sup>5</sup> describe the organs and the sexual process in terms which Kihlmann thinks too confident and premature at the time.

I now pass to the observations made by Kihlmann himself. The macrocysts and paracysts arise together in pairs as terminal swellings of the branches. The hook-like prolongation of the macrocyst becomes fused to the apex of the paracyst, much as described by previous writers; but before the apex of the hook-like process meets the paracyst, a solid septum cuts off its communication with the macrocyst at the point where it leaves the latter.

Hence a mingling of the protoplasm in the two “sexual” organs is impossible, and the open communication described by Tulasne never occurs. From certain changes in the appearance of the protoplasm of the paracyst, at the time when the hook-like prolongation fuses with it, and from the peculiar refractive appearance of the septum, the author is impelled to ask, May not diffusion occur? But, as a matter of observation, the protoplasm in the paracyst does not perceptibly diminish in

<sup>1</sup> Op. cit.

<sup>2</sup> ‘Beitr. zur Morph., &c.,’ R. iv.

<sup>3</sup> ‘Bot. Zeit.,’ 1882; ‘Beitr. zur Entw. einiger Ascomyceten.’

<sup>4</sup> ‘Text-book,’ p. 309.

‘Grundzüge der Systematik, &c.,’ p. 123.



quantity, and soon regains its former appearance, like that of the macrocyst.

The paracyst and macrocyst both become enlarged, and hyphæ bud out from below, enclosing them as described before. Meanwhile, buds appear on the paracyst, which are from the first much thicker than the paraphyses, and are evidently the ascogenous hyphæ.

The macrocyst is therefore an ascogonium, and the paracyst is morphologically an antheridium. Whether these sexual organs are so physiologically is very doubtful.

Comparison with the Collemaceæ<sup>1</sup> suggests that the hook-like prolongation from the macrocyst is really a trichogyne; and it is not impossible that here, as in the Collemaceæ, an extremely small quantity of material may pass into the ascogonium, and the sexual act be physiologically complete also.

Proceeding to compare the foregoing with what we know of other Discomycetes, Kihlmann thinks that the sexual process in *Ascobolus furfuraceus*<sup>2</sup> is still less established than in *Pyronema*. The ascogonium in *Ascobolus* is large and well marked, it is true, but the so-called pollinodium is very little, if at all, different from the ordinary mycelium. Probably in this, as in other *Ascoboli*,<sup>3</sup> the male organ is degenerated. Reference is then made to recent researches on other Discomycetes. Mattiolo's<sup>4</sup> and Brefeld's<sup>5</sup> investigations of *Peziza sclerotiorum* show that the process of reduction has gone still further in this case; even the ascogonium seems to have disappeared. Other *Pezizæ*,<sup>6</sup> so far as the researches allow us to judge, seem to present similar degenerations.

Kihlmann thinks we may probably say that *Pyronema confluens* possesses sharply distinguished sexual organs—at any rate, morphologically. *Ascobolus furfuraceus* probably produces its fructification parthenogenetically, while *Peziza*

<sup>1</sup> Stahl, 'Beiträge zur Entw. der Flechten,' H. i.

<sup>2</sup> Janczewski, 'Bot. Zeit.,' 1871.

<sup>3</sup> E.g. *A. pulcherrimus*; Woronin, 'Beitr. zur Morph.,' R. ii.

<sup>4</sup> 'Nuovo. Giorn. Bot. Ital.,' vol. xiv.

<sup>5</sup> 'Schimmelpilze,' iv.

<sup>6</sup> Cf. Woronin, op. cit., and Tulasne, 'Ann. des Sc. Nat.,' ser. 5, t. vi.

sclerotiorum forms its asci and paraphyses in a purely vegetative manner. At any rate, apogamy must be regarded as occurring in these Discomycetes, and as being attained gradually through a series of forms.

Before referring to other work of Kihlmann's I wish to review an important paper by Fisch,<sup>1</sup> published in 1882. In this are detailed the development of the fructification of several Ascomycetes which form a stroma, in which the proper perithecia are buried, more or less. He is clearly acquainted with the recent researches and speculations of De Bary, and, in fact, worked in his laboratory. Very little has been done with regard to the Fungi mentioned, and so careful a paper as this is especially welcome. The Fungi examined by Fisch are *Polystigma*, *Xylaria*, *Claviceps*, and *Cordiceps*.

*Polystigma* occurs in the leaves of *Prunus*,<sup>2</sup> forming swollen masses. The formation of the ascospores takes place some months after the fall of the leaf. The ascospores, sown in water, produce secondary spores. These send hyphæ through the epidermis of the living leaf, and a mycelium is formed in the intercellular spaces. This breaks down the cells in part, or stimulates them to hypertrophy, and thus the stroma is formed, partly of mycelium, partly of hypertrophied leaf-tissue.<sup>3</sup> Eight weeks after infection, the young spermatogonia appear as knots of hyphæ, which become hollow and abstrict the spermatia.

The young perithecia now arise as small clumps of fine hyphæ, which soon form a sub-globular mass, and in the interior of which a spirally-coiled group of cells represents the ascogonium, and reminds the observer of the ascogonium of the Collemaceæ.<sup>4</sup> This body is somewhat irregular, not evidently attached to a particular part of the mass enveloping it, and it slowly grows as the surrounding perithecial cells multiply.

<sup>1</sup> "Entw. einiger Ascomyceten," 'Bot. Zeit.,' No. 49, 1882.

<sup>2</sup> Cf. Frank, 'Krankheiten der Pflanze,' p. 632.

<sup>3</sup> Cf. Tulasne, 'Selecta Fung. Carp., iii, and De Bary, 'Morph. und Phys. der Pilze, &c.,' p. 8; also Frank, loc. cit.

<sup>4</sup> Stahl, op. cit.

One end of the spiral grows out straight, passes through a stoma, and is clearly of the nature of a "trichogyne." This was frequently seen, and is figured several times. Though spermatia were seen to adhere firmly to the end of the trichogyne, the author could not convince himself that fertilisation took place.

It requires two or three months to complete these processes and the formation of the ripe perithecia. Meanwhile, the trichogyne begins to be disorganised from its free apex inwards. This was confirmed on both the species examined, and the author thinks it is a more pronounced degeneration than the change induced in the trichogyne of *Collema* on fertilisation.

The paraphyses now bud from the base of the perithecium—not from the ascogonium—and soon fill up the space formerly occupied by the dense tissue surrounding the coiled portion of the ascogonium. This tissue meanwhile becomes resorbed, and the few remaining basal cells of the ascogonium—the trichogyne and upper part have disappeared—give rise to asci by budding. All the stages of development are clearly described.

With *Xylaria polymorpha* Fisch was able to clear up the points left undecided by De Bary<sup>1</sup> and Fuisting.<sup>2</sup> The young perithecia arise in the dense stroma as clumps of interwoven hyphæ, in the midst of which a mass of paler cell-rows arises, which are coiled and interwoven into a core or "nucleus." These are the "Woronin's hyphæ" of Fuisting. While these are developing, the outer walls of the perithecium become differentiated. The "Woronin's hyphæ" now break up, first into pieces of one or two cells, and then into a disorganised mass, which soon becomes gelatinous and amorphous.

The paraphyses now spring from the dense mass forming the inner wall of the perithecium. The asci arise from among these, and have therefore nothing to do with the "Woronin's hyphæ," which have disappeared in a slimy mass. Further details offer nothing new.

<sup>1</sup> 'Morph. und Phys.,' pp. 97—99.

<sup>2</sup> 'Bot. Zeit.,' 1867, pp. 303—310.

In *Claviceps*, the young perithecium arises as a mass of small cells, which rapidly divide and form a parenchyma-like mass. A hollow then appears in the interior (remining one in many respects of the cleavage cavity in some animal embryos) by the separation of the cells. The mass now consists of a thick basal portion, over which is a hollow space roofed over in a dome-like manner by the upper cells. There is no trace of an ascogonium or of "Woronin's hyphæ" at any time; the asci arise by budding from the cells forming the floor of the cavity.

*Cordiceps* and other species of both genera agree in the main with what has been described.

In summing up the foregoing, the author points out that while in *Polystigma* we have morphologically the same organs as occur in the *Collemaceæ* (viz. spermatia, trichogyne and ascogonium), no sexual process could be demonstrated. In *Xylaria* the sexual organs—at any rate the ascogonium—is represented morphologically, but has become functionless—it deliquesces and is absorbed before the asci arise; these spring in a purely vegetative manner from the lining walls of the perithecium. It is possible that certain facts observed in *Cucurbitaria* point to the same end.

In *Claviceps* the perithecium is purely apogamous—no trace of an ascogonium occurs, and the asci are produced by a vegetative process of budding from the floor of the perithecium cavity.

Putting together the foregoing facts, and what is known otherwise of the allies of these Fungi, Fisch shows that the compound *Pyrenomycetes* present a series of forms which commence with a complete differentiation of sexual organs (ascogyne, trichogyne, and spermatia), and end in forms which are quite apogamous, and with no trace of sexuality. We may go further than this.

Beginning with *Podosphæra*, in which a sexual process is possibly still recognisable, we trace a series through the simple *Pyrenomycetes* and the *Discomycetes* branching off from these, ending with the completely apogamous *Chætium*<sup>1</sup>

<sup>1</sup> Van Tieghem, 'Compt. rend.,' 1875; and 'Bull. Soc. Bot. Fr.,' 1876.



and Pleospora.<sup>1</sup> In this series there is no place for the composite Ascomycetes and Lichens—for although the sexual process by means of spermatia is only an adaptive form, the difference is too great to fit into the main series. How then do these forms abut on to the main series? Has a sexual process arisen in the Ascomycetes a second time; or did these forms branch off early, and evolve and specialise their peculiar mode of fertilisation from the original type? The first hypothesis cannot be maintained; the second seems highly probable.

We must regard the separation of the sexual organs in the composite Ascomycetes and Lichens as an adaptation, though we cannot say how it came about or served the organisms concerned. It is remarkable that this only occurs in forms which develop a stroma.

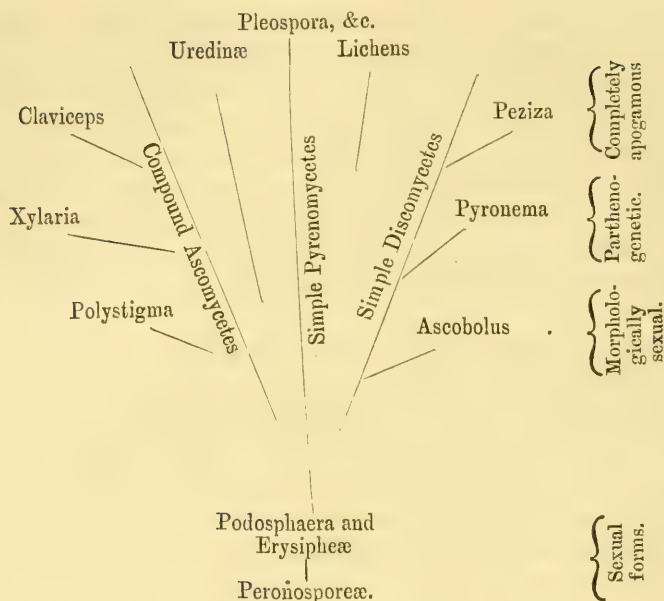
The composite Ascomycetes, therefore, branched off before the sexual process was lost; whether the Lichens came off at the same time is not clear—the latter possibly form more than one series, moreover.

The Discomycetes must also have branched off early from the main series; they form a series in the following forms, gradually culminating in apogamy—*Ascobolus furfuraceus*, *A. pulcherrimus*, &c., *Pyronema confluens*, *Peziza tuberosa*, *Fuckeliana*, *Willkommi* (the latter examined by Fisch).

The Uredineæ must also have come off very early from forms in which sexuality still existed.

As I understand the foregoing, the following scheme fairly expresses the views; it being borne in mind, however, that the lines are not intended to indicate more than the general directions in which descent may be traced. Of course, such a diagram suffers from being drawn on a plane surface. No doubt more than one line should be drawn towards the Lichens.

<sup>1</sup> Zopf, 'Bot. Zeit.,' 1879, p. 73. Bauke, 'Bot. Zeit.,' 1879.



We may now shortly consider the chief results obtained by Kihlmann from cultivations of *Melanospora parasitica*,<sup>1</sup> a pyrenomycetous fungus found associated with, and parasitic upon *Isaria*.

*Isaria* grows upon and destroys insect larvæ, and Kihlmann observed that large quantities of the perithecia of *Melanospora* soon appear with it; the same is true for *Botrytis*—another insect killing fungus. In both cases the sowing of *Melanospora* spores on these Fungi soon resulted in the formation of abundant perithecia.

This, of course, only suggests, but does not prove, the parasitism of the *Melanospora* on the *Isaria*, or *Botrytis*.

Spores of *Melanospora*—whether conidia or ascospores—if sown in water only swell and throw out short tubes, which invariably die off soon. The same happened with all the numerous nutritive solutions tried. These solutions were varied not only as to composition, but also as to concentration, &c.

<sup>1</sup> Op. cit.

If a spore germinates in the neighbourhood of a living hypha of *Isaria*, however, the germinal tube fixes upon the *Isaria* hypha and at once emits more tubes, which are thicker and more vigorous than before. If the germinal tube from the *Melanospora* spore comes within a certain maximum distance from a branch of *Isaria* its apex grows directly towards the latter until a union is effected.<sup>1</sup> This was observed and confirmed several times. All the above facts are generally true of the conidia also; and *Botrytis* may be substituted for *Isaria* as the host plant.

After about eight days the above processes have resulted in the formation of a vigorous mycelium and the formation of young perithecia. The perithecium commences by the development of a lateral branchlet, which becomes coiled two or three times, and divided by a few septa; this is the ascogonium. It frequently resembles that of *Ascobolus*.

Thinner hyphal branches now spring from below the ascogonium, and envelope it by applying themselves closely to it, and branching and dividing; although one of these may grow out more rapidly at first, it does not seem to more than hint at an antheridial branch. But very often two or more arise together, and others soon follow in all cases.

None of these branches copulate with the ascogonium. Although *Isaria* branches may be close to and serve to nourish the hyphæ producing the fructification, there is no doubt whatever that only the hyphæ from the *Melanospora* enter into the constitution of the fructification.

The details of the development of the perithecium wall from the enveloping hyphæ are interesting, but present nothing essentially new, and need not be described here.

Of the four or five cells into which the coiled ascogonium is divided, the cell below the apex forms the ascogenous tissue. The terminal cell above it becomes disorganised; it is sterile,

<sup>1</sup> Cf. my description of the behaviour of a hypha of *Pythium gracile*, 'Quart. Journ. Mic. Sc.,' October, 1883, p. 504; and also the remarks below on the behaviour of *Spirogyra* in conjugation.

and soon disappears. Its immediate neighbour (i. e. the cell below the apex) becomes cut up by numerous septa in all planes, and forms an ascogenous core of parenchymatous tissue. As this occurs, the internal layers of the now dense envelope—produced by repeated ramifications and divisions of the interweaving hyphæ of the enveloping branches—become disorganised, deliquescent, and evidently then serve as nourishment for the cells of the ascogenous core.<sup>1</sup> The proximal cells (i. e. those cells between the ascogenous cells and the mycelium) of the ascogonium also disappear, and the enveloping layers around them become elongated to form the long neck of the perithecium. It thus comes about that the free apex of the ripe perithecium corresponds to what was the attached end of the ascogonium.

The walls meanwhile become coloured deep brown, and rudimentary paraphyses spring from their internal layers. The asci arise from the colourless cells of the ascogenous core, in which a cavity is produced by the tangential growth of its peripheral cells.

In the concluding remarks stress is again laid upon the fact that we cannot speak of an antheridium here; the antheridia have degenerated to mere vegetative hyphæ, and the ascogenous core produces its asci without any sexual process whatever. A single cell produces the asci. In most respects this agrees with Gilkinet's description of the analogous processes in *Sordaria fimicola*.<sup>2</sup> But in *Sordaria*, Gilkinet finds one enveloping hypha apply itself to the ascogonium before the rest, though he could not decide that copulation took place.<sup>3</sup> Kihlmann denies that this can be termed an "antheridium." He regards *Melanospora* as somewhat midway between those *Erysipheæ* which, like *Eurotium* and *Podosphaera*, have the sexual organs at least morphologically present, and the truly

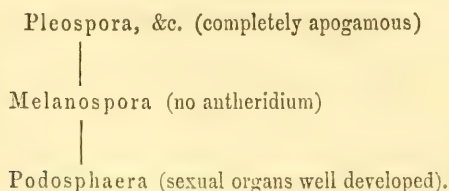
<sup>1</sup> Cf. my description of the development of the perithecium in *Meliola*, *Phil. Trans.*, 1883, p. 592, &c.

<sup>2</sup> 'Bull. Acad. r. de Belgique,' ser. 2, t. xxxvii.

<sup>3</sup> Cf. also De Bary and Woronin, 'Beitr. zur Morph. und Phys. der Pilze, &c.,' 1870, R. iii.



apogamous *Pyrenomyces*, *Chætomium*<sup>1</sup> and *Pleospora*.<sup>2</sup> We may no doubt fairly represent these views in such a diagram as the following :



I now proceed to notice a further contribution to our knowledge of the *Ascomycetes*, by Eidam.<sup>3</sup> Passing over his description of *Eremascus albus*, a new species and genus (in which the process of conjugation is, however, strongly suggestive of the *Zygosporeæ*), we may briefly notice the general absence of any recognisable process of fertilisation, though an ascogonium is always present, and Eidam seems to regard one of the enveloping branches as an antheridium—morphologically at least.

This observer has studied the development of the perithecium in *Chætomium*. The ascogonium arises as an isolated coiled branch; fine branched hyphæ then envelope it. The tufts of fine hyphæ may, however, arise independently of ascogonia also. Other cases occur where the ascogonia show no traces of anything but vegetative budding from the hyphæ. He regards it as possible that a sexual process occurs in the cases first described, and that all stages of degeneration to complete apogamy occur. Questions of nutrition seem to affect this matter.

It must be allowed that the figures do not establish this, however, and it seems very questionable if any antheridial branch whatever can be distinguished.

*Sterigmatocystis nidulans* is a new species of *Fungi* allied to *Aspergillus* and *Eurotium*. An interesting de-

<sup>1</sup> Zopf. 'Nova Acta, Leop. Car. Akad.,' Bd. xlii.

<sup>2</sup> Bauke, 'Bot. Zeit.,' 1877.

<sup>3</sup> Cohn's 'Beitr. zur Biologie, &c.,' B. iii, H. iii.

scription of its capability of producing pathological changes when injected into the blood of animals can only be adverted to here. The fructification occurs embedded in a sort of stroma of hyphæ, interwoven into dense cushions, the peculiarities of which need not be detailed.

The simple ascogonium is enveloped by a hypha ("antheridial branch"), which soon becomes septate and branched, and forms in the perithecium wall. The ascogonium forms a multicellular core, from which the asci arise. No fertilisation is shown to occur.

*Helicosporangium parasiticum* was also thoroughly studied. Here, again, we have simple ascogonia enveloped gradually by so-called antheridial branches. The author does not make quite clear, however, what are the ultimate fates of the several parts. One central cell becomes filled with spores, but Eidam differs from Karsten as to the meaning of this. He also denies that *Helicosporangium* is parasitic.

A closely-allied form is *Papulospora*, which agrees with the latter in forming the peculiar masses of cells which seem to represent young perithecia. It is difficult to avoid the conclusion that Eidam has either figured ill-nourished specimens—which appears unlikely—or that some unknown conditions would have caused the bulbil-like bodies to form perithecia.<sup>1</sup> Be this as it may, the bodies in question form no asci, but "germinate" like compound spores. The great variability in the formation of the spores and fructification in these Fungi supports the suggestion ventured above, and there can be little doubt that Eidam has opened a question of great importance, and succeeded in showing that variability occurs in these processes—whether due to conditions of temperature, nutrition, moisture, &c., or not, cannot yet be determined. There are facts to support this, and indeed Eidam has shown this in some examples.

<sup>1</sup> I have drawings of somewhat similar bodies from an unknown fungus, which cannot as yet be made to develop further: they appear to be young perithecia, but they germinate directly, like gemmæ, when the conditions are favorable.

Concerning *Eremascus*, where a true conjugation takes place between the apices of two similar hyphæ coiled round one another like a double screw, it is not easy to see why the product of the sexual act (a globular body situated between the conjugating apices) should not rather be termed a zygospore than an ascus. The fact of its containing eight "spores" instead of one, is no more peculiar than in the case of the *Saprolegniæ*, where an oogonium may contain one to twelve or many more oospheres. Eidam recognises the general similarity to *Zygosporeæ*, but gives no adequate reasons for choosing the name "ascus" in preference to "zygospore." The eight-spored body would be an extremely anomalous ascus; but it is impossible to decide the matter until the asexual spores are discovered. It is interesting to note, however, that the so-called "asci" arise parthenogenetically in rare cases.

The main results of Eidam's observations go to prove that in apogamous forms there may be more or less indications of certain rudimentary organs—antherial branches (?)—but they do not seem to establish his conclusions that sexuality exists in these forms. Of course it is open to imagine that the sexual act comes in now and again, as Eidam suggests, but no one acquainted with the facts will lay stress on this supposition.

If we now turn from the *Ascomycetes* to the other groups of Fungi, the chief papers published lately are not very numerous.

The most important, probably, is Woronin's memoir on the *Ustilagineæ*,<sup>1</sup> and his description of the hitherto little-understood *Tubercinia trientalis*. Woronin devoted much time to this investigation, commenced sixteen years ago. We may shortly summarise the life-history as follows:

In May and June the under side of *Trientalis* leaves are apt to be covered with white patches. These consist of the colourless conidia,<sup>2</sup> supported on long hyphæ, much like those of *Ramularia*, *Peronospora*, and others. These conidia-bearing hyphæ spring from a mycelium in the leaves. In the

<sup>1</sup> 'Abhandl. Senk. Nat. f. Gesellschaft,' B. xii, H. iv, 1881.

<sup>2</sup> These are true conidia, homologous with those of *Ascomycetes*, and have nothing to do with the ordinary spores and "sporidia."

mesophyll are abundance of the usual brown ustilaginous spores—very like those of *Sorosporium*, &c.—in dense clusters.

In the autumn the *Trientalis* plants are found spotted with black patches. These are due to the densely-clustered brown compacted spores, as before, but no conidia occur now. The pyriform colourless conidium germinates on the leaf surface; the germinal tube bores its way in, grows to a mycelium which ramifies between the cells, and sends branched haustoria into their cavities.

At certain points on the mycelium arise lateral branchlets, which superficially resemble ascogonia, at least in some cases; these—single or several together—become enveloped by fine hyphæ, and soon present the appearance of a dense grape-like cluster of spores embedded in the interwoven mass of fine hyphæ. The investment becomes disorganised as the clusters of spores turn yellow, and then brown, and acquire thick coats. The cluster of spores germinates as a whole, putting out tubes (“promycelia”) at the end of which the crown of “sporidia” appear according to the type of *Tilletia*. These oval sporidia may also “copulate” in pairs in the well-known manner; but this often does not occur, and it seems to be an unimportant point, not affecting the future of the sporidia or their progeny at all. Secondary and tertiary sporidia may arise from the primary sporidia by budding.

After sowing the brown spores on young plants of *Trientalis*, still level with the ground or nearly so, the mycelium arises in the seedling, and, as soon as the leaves unfold, produces the white conidia externally and the brown compound spores internally. This is no doubt the best established case of the existence of two generations (producing conidia and spores respectively) that we as yet know of in this group; it is true, it is not the only case, for we have the same thing in *Entyloma*.<sup>1</sup> *Tubercinia* also agrees with the others in being antœcious, i. e. in passing its whole life on the same host plant. Woronin is of opinion, however, that “a whole series of hete-

<sup>1</sup> De Bary, ‘Bot. Zeit.,’ 1874, p. 81.



rœcious forms" will be discovered among Ustilagineæ; whether this is an inference inspired by facts not yet published does not appear.

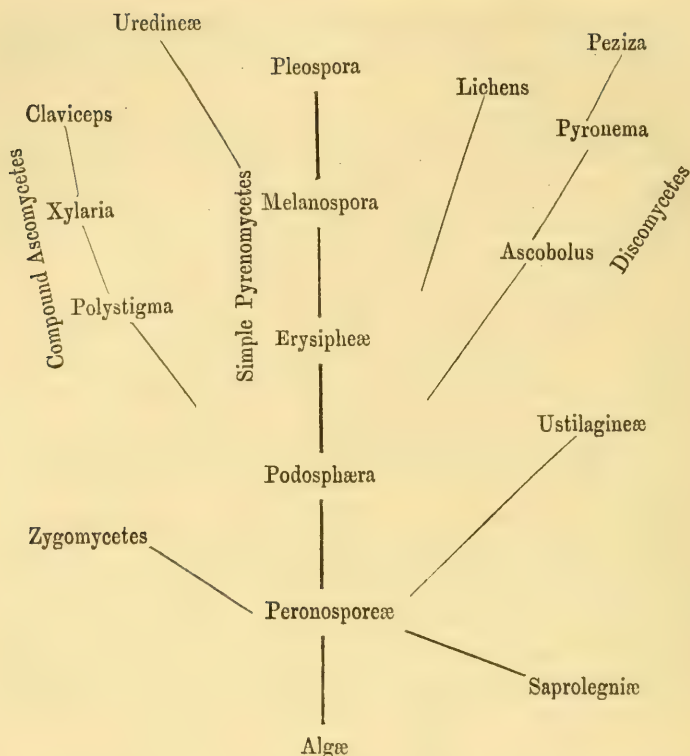
Of the remainder of Woronin's magnificent paper nothing need be said here; and space does not admit of our referring more in detail to the other papers lately published on the Ustilagineæ by Brefeld<sup>1</sup> and Max Cornu,<sup>2</sup> which, moreover, contain little of importance for our present purpose.

In the foregoing part of this essay I have collected a mass of evidence tending to support the view definitely stated by De Bary, to the effect that, as we proceed along the main lines from the lower to the higher Fungi, the sexual process and sexual organs gradually become less and less evident, and at length disappear altogether, and the fructification arises by apogamy.

If we try to follow the various groups of Fungi phylogenetically, there can be no doubt that they may be placed, on morphological grounds, much as De Bary has grouped them; and if, taking into account what has been said above, we attempt to arrange the smaller groups as branches of the larger ones, we shall, I think, arrive at a scheme not very different from that annexed.

<sup>1</sup> 'Schimmelpilze,' iv.

<sup>2</sup> 'Ann. des Sc. Nat.'



It must be borne in mind that we confine ourselves strictly to the evidence derived from the study of living forms—it may or may not be that numerous primary or ancestral forms, long since disappeared, would lead us to different conclusions, as the imagination of such led Brefeld to different views; but the only true method is to adhere to what we know as the basis of our plans for knowledge to come.

It must be allowed at the outset that we know very few forms accurately or thoroughly, and there are therefore almost endless possibilities in the future. Keeping these cautions in mind, however, we need not fear to point out whatever points of general significance can be obtained from our present knowledge.

The first and most important fact with regard to the scheme is that if we pay regard to the terminal members of most of the main branches, we notice that they are all or nearly all parasitic forms, or, at any rate, include such forms.

The higher Ascomycetes offer us the following examples from different branches, the Lichens, Pleosporas, &c., Claviceps, &c., and *Peziza sclerotioides*.

Then come the Uredineæ (we need not necessarily imagine the Tremellas and Basidiomycetes as derived from the highest Uredineæ; the evidence does not decide this), all strongly parasitic.

The Ustilagineæ of course are parasites par excellence, and they terminate a side series.

We have still two main groups to deal with—the Saprolegniæ (which, so far as known, are mostly saprophytes) and the Zygosporæ, which are also generally saprophytes. Our very imperfect knowledge of the Basidiomycetes will be cited as an excuse for putting them aside in what follows: I do not for a moment under-value what we do know of them, but, as the sequel will show, their present position becomes more and more anomalous, if we really know the entire life-history. Of course we have no right to quarrel with the evidence, but the story of these Fungi, as told at present, completely negatives their being included in the scheme to follow, and we must therefore neglect them for the moment, merely reminding the reader that some of them are parasitic.

Neglecting the Basidiomycetes, then, we may proceed to note that not only are the terminal groups of the series named usually parasites, but that it is just in those groups which are most intensely parasitic that least hope of our discovering sexual organs exists. In the Zygomycetes, on the other hand, we have the sexual process and typical saprophytic habits together, while in the Saprolegniæ the case seems doubtful.

Looking still more closely into the matter, it appears as if the absence or presence of sexual organs (or their rudiments) rises or falls with the nature of the parasitism or saprophytism

displayed. In the Saprolegniæ, for instance, the Fungi may probably be looked upon as very highly nourished by the decomposing proteids of animals.<sup>1</sup> Their sexual organs seem to be present in most cases, but functionless.

In the Zygomycetes, which are essentially saprophytes on decaying vegetable matter, &c., or parasitic on one another—and may probably be regarded as not so highly nourished—we find the sexual organs functionally perfect, though very simple in character.

In the Ustilagineæ we meet with parasitism of a peculiarly high order, so to speak. The fungus not only robs its host, but has in most cases curiously adapted its life to the habits of the latter, using it rather as a slave than as a victim to be destroyed forthwith.

The same is true for the highly-organised Uredineæ (*Æcidiumycetes*), and we here meet with the highest adaptation of all, heteroecism. But in these two groups the search for sexual organs has proved utterly futile (if we except the so-called “copulation” of the “sporidia” in Ustilagineæ, which cannot be regarded as an essential process, or as sexual in the above meaning).

Again, if we proceed upwards from the Erysipheæ, which are epiphytes—adapting themselves to parasitic habits of that special kind which leads to life in the interior of temporary organs like leaves—through the Ascomycetes, we find, speaking generally, more and more tendency towards close and specially adapted parasitism, ending in the Lichens, the parasitic Pezizas, forms like the *Pleosporas*, &c., and especially *Claviceps*.

Now it is at least remarkable that no trace of sexual organs has yet been found in the higher Lichens—i. e. in those forms in which the fungus makes a particularly well-regulated use of its slave-like host, which is an Alga containing chlorophyll. Krabbe<sup>2</sup> considers that in *Sphyridium* the fructification arises

<sup>1</sup> If not, indeed, by living flesh. Cf. Prof. Huxley, ‘Quart. Journ. Mic. Sc.,’ 1882.

<sup>2</sup> ‘Bot. Zeitg.,’ February, 1882, No. 5.



independently of any process of fertilisation, and my own observations on *Strigula complanata*<sup>1</sup> lead to the same conclusion. It will be noted that in the beautiful case demonstrated by Stahl,<sup>2</sup> the host is a blue-green Alga, and the parasitism may well be considered as lower in many respects. Moreover, it is by no means certain that the Lichens represent one group.

In *Claviceps purpurea* we have an excellent example of the highly-developed parasitism referred to. The ravages of the parasitic mycelium seem to be confined to one organ of the host—the young fruit—and we have seen from Fisch's researches that the asci arise in the stromata, developed later, in a purely vegetative manner.

Our knowledge of the large group of the simpler Pyrenomycetes does not enable us to make a generalisation of very much value; but it is significant for our present purpose that the apogamous *Pleospora*, for instance, is parasitic during the early stages of its life, and, like so many of its allies, adapts its cycles to those of its host, producing a large stock of asexual conidia on the living leaves, and using up their contents before falling, to complete the development of the asci, &c., on the ground. It is scarcely necessary to remind the reader how great an advantage accrues to these higher parasites, when they scatter immense quantities of spores from leaf to leaf of the living tree. That their perfect "fruits" should be formed later, when the mycelium has gathered up all the material possible, is quite in accordance with what occurs in the formation of stromata, sclerotia, and masses of hyphæ (often with haustoria) around the young perithecia in other cases.

The same is generally true for such Discomycetes as *Peziza sclerotioides*,<sup>3</sup> *P. Fuckeliana*, and other parasitic *Pezizæ*; and it will be remembered that it is in these forms that De Bary and others failed to find any traces of sexuality, thus

<sup>1</sup> 'Linn. Trans.,' ser. 2, 'Bot.,' vol. ii, 1884.

<sup>2</sup> *Op. cit.*

<sup>3</sup> Frank, 'Krankheiten der Pflanze,' p. 531, &c.

placing them in strong contrast to such as *Ascobolus* (according to Janckweski's researches), unless intermediate forms like *Pyronema* and the saprophytic *Pezizas* are compared also.

Enough has now been said to show that there is at least strong reason for believing that a connection exists between the mode of life of a given Fungus and the extent to which it is apogamous. It will no doubt be suggested that there are still cases where this view seems at variance with the facts. Without wishing in any way to strain matters at this point, it may be noted that we really know very little of the mode of life of very many Fungi, and that the terms saprophyte and parasite are used somewhat loosely. This being admitted, it may happen that further knowledge will strengthen the connection spoken of.

We are at least assured that profound differences exist—in degree, at any rate—between the saprophytism of a *Mucor* growing in a solution of horse-dung, and of a *Pythium* developing its fructification in the rotting parenchyma of a plant which it has previously killed.

There is also an equally striking difference between the parasitism of an epiphyte like *Erysiphe* and that of a highly-specialised *Æcidium* like *Puccinia*. But I would insist upon more than this. It is not only in the mode of attacking or living upon the substratum that one fungus differs from another; differences as to the kinds and quantities of the various matters absorbed must also exist, and a *Uredine* in a leaf no doubt obtains different food (and in a different way) from that taken by *Claviceps* in a grain of rye, or *Ustilago* in a hypertrophied swollen stem of *Zea Mays*. That these differences may be very important—though we do not know exactly in what they consist—is fully demonstrated in cases of heterœcism.

I have already pointed out that the coexistence of apogamy (or the total suppression of sexual organs) and parasitism is noticeable especially in the highly specialised parasites. In forms which, like the majority of the parasitic *Peronosporæ* and *Zygomycetes* (e.g. *Peptocephalis*), are nevertheless

provided with sexual organs, which, so far as we can see, are quite like those found in the saprophytic forms, we have two points to notice. First, these forms are close to the parent stock in phylogeny—i. e. they are not much modified from the type of *Pythium* itself, which (as a comparison with *Vaucheria* shows), is no doubt derived from algal ancestors, and with strongly inherited sexuality. Secondly, such forms are probably not so highly parasitic as is commonly supposed. I do not mean to say that their living hosts are not robbed by them; but it is significant that the *Peronosporæ* are often saprophytes, and that even the most parasitic forms break down the parenchyma of the hosts to a rotting, fetid mass, on which they then flourish. Moreover, they are aided by bacteria in this process. In addition to this they are apt to be omnivorous. I have cultivated *Pythium De Baryanum*<sup>1</sup> on the most various substances, as well as on more than a dozen widely different living plants.

In all these cases the parasite appears to flourish in a variety of substrata, and it has not got over the clumsy habit of destroying its host forthwith. If we compare the highly developed, almost intelligent, parasitism of a higher Ascomycete or Uredine with this, it will be understood what I mean by specialised parasitism. Instead of clumsily destroying its host (like *Phytophthora infestans* does the potato), a *Puccinia* is adapted to live in isolated patches of carefully-sheltered leaf tissue, ramifying in the lacunæ filled with oxygenated air and aqueous vapour. Here it taps the cells as they manufacture organised substances in the sunlight, taxing them not too much for their strength,<sup>2</sup> and its mycelium keeping near the stomata. Its spores are then protruded in centrifugal succession, and shaken off from their advantageously high position on to other leaves, &c. All such adaptations must imply long periods of descent (and the fungus is therefore much further from the parent stock in the phylogenetic scheme), during which even the

<sup>1</sup> Cf. also De Bary op. cit., and this Journal, 1883.

<sup>2</sup> Many Uredinæ appear to do no injury at all, unless in large amount and for a long time—i. e. the host can pay the tax easily.

strong hereditary tendency to produce sexual organs, &c., might become lost, if such organs for any reason became superfluous.

This, however, brings us at once to the last object of the present essay; and I propose to show that it is probable that the sexuality of the higher Fungi has disappeared, because its purpose has been equally well or better attained otherwise than by means of sexual organs.

Preliminary to this it will be necessary to be quite clear as to what sexual organs and the sexual process essentially are.

The two points common to all the cases of sexual reproduction which have been directly observed are the following:

1. A larger or smaller quantity of protoplasmic material passes from one portion (the male organ) of the same or another individual, into the protoplasm contained in another portion (the female organ).

2. The protoplasm contained in the female organ therefore becomes capable of further development; either at once, or, more generally, after undergoing a period of rest.

It is not necessary to quote the numerous cases of observed analogies between the sexual reproduction of animals and plants; but will suffice to note that the essential in the sexual process is always the addition of a portion of protoplasm from the male, to the protoplasm of the female.

But this is not all. It is now well established in embryology that the normal ovum, or female mass of protoplasm, is incapable of further development until it has received the protoplasm of the male; that the latter, in fact, incites the former to further development. In many cases, indeed, the protoplasm of the egg or ovum gets rid of a small portion of its substance, as the "polar bodies," as if to make room (so to speak) for the substance coming to it from the male.<sup>1</sup>

While in the higher organisms we can distinguish the male elements—spermatozoa, antherozoids, &c., only in so far that they are much smaller and more numerous than those of the female organs; we find that in the lower forms of life even

<sup>1</sup> That something of the same kind takes place in the Saprolegniæ is suggested in my paper on this group, 'Quart. Journ. Mic. Sc.,' 1883.



this difference in size is absent, and there is absolutely no safe criterion to determine which of the two conjugating masses of protoplasm is to be regarded as male and which as female.

Nevertheless, if we consider cases such as are afforded by the Fungi, we are certainly on safe ground when we call the antheridium of *Pythium* a male organ, and the oogonium of the same a female organ. The protoplasm contained in the former is itself incapable of further development, but normally passes over into the protoplasm (oosphere) contained in the latter; the oosphere is then—i. e. after fertilization—capable of further development.

This "further development," however, is nothing more than growth; and, what is more, growth according to the same laws as affected the parent plant which produced the sexual organs. In cases where the plant is divided into cells, this growth or germination of the oospore commences with division into a number of cells.

The outcome of all we know of these matters leads to the conviction that we have in the germination or development of an oospore—and the same is true for an egg, &c., the terms being different—simply a renewal of the growth of the organism; and from this and other convictions follows the result that the formation of an oosphere, although it may take place after an accumulation of large quantities of food, implies a condition of weariness—if the term may be allowed—on the part of the protoplasm for the time being. No doubt the molecular energy of the protoplasm forming the oosphere, is less than that of the rest of the plant for the time being; the access of the antherozoid or male protoplasm, however, reinvigorates the sluggish mass, and renewed life ensues. This may require some time, however, and we may possibly not be far wrong if we imagine that interval to be occupied in molecular rearrangements in the mass.

But, although we can sum up the foregoing by saying that, after a time, protoplasm requires re-invigorating by the addition of fresh protoplasm from another source, it is extremely

improbable that the protoplasm of the male and female organs is at all similar.

While we have reasons for believing that the mass of an oosphere consists in the main of protoplasm such as occurs in any cell capable of growth, it would be absurd to suppose that the protoplasm of the male element is of the same nature. There is, moreover, strong evidence to support the opposite view, that the protoplasm of the male and the essential protoplasm of the female differ extremely.

Anyone who reads Strasburger's description of the process of fertilization in the ferns,<sup>1</sup> cannot fail to be struck with the peculiar behaviour of the antherozoids as soon as they come within a certain distance of the oosphere. It seems impossible to avoid the inference that the oosphere in some way attracts the spermatozoids. A similar phenomenon is described by Juranyi in the fertilization of *Cedogonium*,<sup>2</sup> where the relatively large antherozoid forces its way through an aperture too small for it, in order to reach the attracting oosphere.

With such phenomena may be compared the case of *Spirogyra* and other *Conjugatæ*, where, as is well known, the cells of filaments which are laid parallel to one another, and within a certain distance of one another, put forth conjugating tubes which meet in the middle; or neighbouring cells conjugate.

In the *Peronosporæ*, again, the oosphere appears not only to attract the antheridium, but even to induce its formation from a neighbouring hypha;<sup>3</sup> and other cases may be cited, all tending to show that some important difference exists between the protoplasm of the two sexual organs.

It does not concern us here to give any opinion on De Bary's suggestion that profound chemical differences exist, and affect the environment; or on Sach's recently expressed view<sup>4</sup> as to the analogies between ferment actions and fertilisation.

<sup>1</sup> 'Jahrb. f. wiss. Bot.,' vii.

<sup>2</sup> 'Jahrb. f. wiss. Bot.,' ix.

<sup>3</sup> De Bary, 'Beitr. zur Morph. und Phys.,' iv.

<sup>4</sup> 'Vorlesungen über Pflanzenphysiologie,' p. 491.

Enough for our purpose that the knowledge we possess goes to show that sexual reproduction essentially consists in the re-vigoration of a sluggish mass of protoplasm, by the addition of another and different mass of protoplasm. That an advantage is often attained by the latter mass coming from a distant source, is sufficiently evident from what we know of cross-fertilisation generally.

It now remains to be seen if we can throw any light on the curious disappearance of sexual organs and sexuality in the Fungi—curious, because the sexual process appears to be all but universal in all organisms excepting the very lowest.

A hypothesis which suggests itself, and which Eidam favours, and which is certainly supported by some analogies, is to the effect that the apogamous Fungi are not always apogamous. We know that many forms only produce their sexual organs at comparatively long and rare intervals. The *Mucors*, for instance, may be propagated through numerous generations by means of the asexual spores; the sexual organs only arising now and again under favorable conditions.

Accepting that the sexual process consists essentially in a re-invigoration of the protoplasm of the organism, may it not be that one sexual act is effective through long periods and many generations? Such a view is supported by the known cases of parthenogenesis in other plants, and would explain such cases as the *Saprolegniæ*, if it were placed beyond doubt that protoplasm does occasionally pass through the “fertilising tubes” to the oospheres.

Moreover, the cases of polyembryony—where several embryos arise in an embryo sac, although only one oosphere is fertilised—favour the view that the effect of fertilisation may be extensive; and we cannot doubt that such is the case where adventitious covering branches arise after the conjugation of certain *Mucorini* (e.g. *Mortierella*), and in the *Orchideæ*, where fertilisation or even the mere growth of the pollen tube affects the whole flower.

In other cases, however, great difficulties are experienced. It is not easy to conceive how fertilisation in a distant past has

transmitted its effects through countless generations to the individual plants of *Chara crinita* which now reproduce without any sexual act at all. And the same is true for other cases.

There is one fact apparently universal in sexual reproduction ; it does not take place until a large quantity of material is either accumulated, or is in some way placed at the disposal of the sexual organs. If these sexual organs are to be looked upon as specialised to secrete the sexual elements, or to sort the substances of which they consist, as it were, this may be of importance.

It must be allowed that no satisfactory theory exists, however, to account for the gradual disappearance, first of sexuality, and then of even the morphologically represented sexual organs in the Fungi ; and any attempt to explain the matter seems to involve more than one vicious assumption.

The sexual act, however, consisting simply or mainly in the re-invigoration of protoplasm by the addition of protoplasm of a different nature (though we do not know the kind or limit of difference) from a distance, it may be that an explanation of what occurs in the Fungi is afforded by their mode of life. I have already pointed out that the Fungi in which sexual organs seem to be most certainly absent are those which are most highly specialised as parasites. Now, we have every reason to believe, first that parasitism is a matter of degree, and secondly that the most highly specialised form of parasitism consists in directly obtaining those contents of the cells of the host which are chemically most complex, and therefore contain most energy.

I need not dwell on the degrees of parasitism exemplified by plants which merely rob their hosts of space or moisture, or which have obtained a hold so intimate that they break it up and feed on the rotting débris, but may at once pass on to consider a few consequences which follow from the mode of life of those highly specialised parasites which have become so closely adapted to their host, that they exist for a time as all but an organic part of its tissues and substance.



It can scarcely be doubted that the protoplasm of a higher plant, such as a phanerogam, differs from that of a lower cryptogam in being capable of doing more work; and that the great advantage derived by a parasitic Fungus which has its life so adapted that it can tax the cells of a phanerogamous host plant, is that it obtains its food materials in a condition more nearly approaching that of its own substance, than would be the case if it had to work these materials up from inorganic matters.

Now it seems not improbable that the protoplasmic substance of a higher phanerogam may contain so much energy, that it can not only supply the vegetative mycelium of a parasitic fungus with all that it requires for its immediate growth, but also suffices to enable that fungus to store up enough energy in its asexual or apogamous spores to last until the next generation of the fungus gains its hold-fast on another (and it may be distant) source of life-giving substance.

Let us take the case of a uredinous fungus parasitic in the leaves of a phanerogam. We know that the substances necessary for the whole growth of the phanerogam are formed in the cells of the leaf; not only so, the matters which eventually find their place in the reproductive organs must be formed there also, potentially at least. The leaf of a phanerogam so attacked, moreover, is able to support the parasitic fungus for a long time uninjured, as I have convinced myself by experiment, and there can be no doubt that substances pass into the fungus which would normally have passed into other parts of the host plant itself. Since these substances serve to support the comparatively enormous display of energy evinced in the growth, &c., of the phanerogam; we need not be surprised if they can also provide in addition for the parasite for the time being.

But we may imagine even this to fail after a time. Without speculating as to the possible differences effective to a mycelium which obtains enough to produce spores on one leaf, which, germinating on another, produce a mycelium which derives an advantage corresponding to that obtained by plants

when cross fertilised—we may suppose that at length the Fungus derives too little benefit to be able to go on, or the season during which the host plant flourishes is drawing to an end.

No doubt we have in heterœcism the salvation of such a Fungus. Not only is it carried through a dangerous period, by seeking relief at the hands of a second host, but—and which I believe to be far more important—it obtains re-invigoration by the new protoplasm with which it comes in contact. We may not inaptly compare the sojourn of the Fungus on its second host, to a trip to the seaside, where the weary and enfeebled organism enjoys fresh diet and associations for a time, which in their turn pall and prepare the recipients to renew old modes of life.

We have seen that the disappearance of the sexual organs, leading to apogamy, commences especially in the lower Ascomycetes, and it may be more than a coincidence that epiphytic forms, which show a tendency to produce one kind of spore while on the living leaf and develop their asci on the fallen leaf are common here; such forms suggest how the parasitism and heterœcism of higher forms may have begun, and it is remarkable that the apogamy becomes more and more complete as we ascend through the latter.

It is not pretended that the hypothesis embodied above at once explains all the cases possible, and it will be well to state a few of the greater difficulties at once. The Basidiomycetes I shall not dwell upon, since our knowledge of them is still very imperfect. The few cases of parasitic Basidiomycetes known can hardly be cited as supporting the view adduced; and if it turns out that all the forms are as utterly devoid of sexuality as Brefeld's *Coprinus*, and that no other generation exists than the one now known, it will be difficult or impossible to reconcile the facts, and the coincidences referred to in this essay may have to be accepted as coincidences only.

Apart from this, the difficulty must suggest itself to many that there are parasitic Fungi—such as the *Peronosporæ*—which nevertheless develop the sexual organs in the condition

typical and perfect for the group to which they belong. I have already referred to the fact that many of these forms are really saprophytes, and that others break down and destroy the tissues of their hosts—clumsily killing their prey and then feeding on the rotten mass—and have pointed out that this is a much less specialised form of parasitism than that of the higher Fungi and Ustilagineæ. It is true we do not know much about the nature of the food which these Fungi take from the host; but there is evidence to show that it is of the nature of fermenting sap, and therefore possibly contains far less energy than the substances absorbed by the higher parasite Fungi. There are two other points which may also be of importance.

The Peronosporæ are almost certainly descended directly from Algæ which had already won and established strongly-marked sexuality. This would probably be lost only after a long time; for we have every reason to suppose that inherited sexual tendencies are among the last to disappear in the modified descendants of organisms.

Nevertheless, and this is the second point, the sexuality shows signs of disappearance in extreme members, even within the groups of the Peronosporæ. De Bary<sup>1</sup> shows that in *Phytophthora* and *Peronospora* there is a less evident passage over of protoplasm from the antheridium to the oosphere than in *Pythium*; and that in some cases, indeed, the quantity passing over is too small to be observed. I will not attempt to lay stress on the coincidence that in *Phytophthora infestans* (the fungus of the potato disease) no sexual act has as yet been discovered.

Another obvious objection may be raised as follows: The Saprolegniæ are in the main saprophytes, and yet they are said to be advanced towards apogamy—parthenogenetic, at any rate. The answer may be that they are saprophytic chiefly on animal protoplasm which contains more potential energy than does vegetable protoplasm. At the same time, some Saprolegniæ are parasitic on plants, and *S. ferax* now appears to be parasitic on fish.<sup>2</sup>

<sup>1</sup> 'Beitr.,' iv, p. 72.

<sup>2</sup> Prof. Huxley, loc. cit.

Among the Zygomycetes, again, we meet with parasitic forms in which the very simple sexual organs and process are, so far as we know, as typically perfect as in the other members of the group. The reply here is the same as in the case of the Peronosporæ. The Mucors must be an older group than Piptocephalis and others which are parasitic upon them. Hence we may assume that the inherited sexuality is too strong to have been replaced by the effects of admixture of the protoplasm of the Mucor, which, moreover, is probably not very different, and can scarcely be considered as provided with more energy. A similar argument may apply to the Lichens. The higher forms are specialised parasites on green Algæ, which must be able to supply substances containing great potential energy, and no traces of sexuality are found in them. In the Collema-cæ, however, where sexual organs occur, the fungus is associated with a very low form of Alga, one of the Cyanophyceæ, and appears rather to be feeding upon the diffuent matters around the algal cells, than strictly parasitic on the Alga proper. This is so much the case that, as is well known, some lichenologists have doubted whether to rank the Collemaceæ with Lichens at all; and all observers must agree that it is difficult to decide when a mass of Nostoc is to be regarded as all Alga or passing into the state of Collema. I remember cases in my time at Cambridge, when I observed patches of Nostoc on the roadside at Shelford, and patches of Collema some distance away. At points between, there were patches of Nostoc in various stages of transition between the two. In Ceylon, again, I have observed masses of Rivularia with fungoid hyphæ associated at least as definitely as in these cases, and the same occurs in masses of Glæocapsa in greenhouses. I do not attempt or wish to cast a doubt on the lichen character of the Collemaceæ; I merely point out that, as in the case of other parasitic Fungi, the Ascomycetes of the Lichens exhibit gradations of parasitism from mere association to highly adaptive parasitism, where the Fungus has learned (so to speak) to use its host as a slave.



he most serious objections to the above hypothesis will probably occur to those who draw conclusions from the life-history of imperfectly known forms. Without wishing to disarm any criticism whatever, I would mention two points to be borne in mind in this connection.

Many Fungi are known to be capable of adapting themselves to widely different modes of life, and it is extremely difficult to say how far they are parasitic or saprophytic. Leaving the Bacteria alone, I need only mention Koch's experiments with species of *Mucor* and *Aspergillus*, and Eidam's observations on *Sterigmatocystis*:<sup>1</sup> these Fungi were found to be pathogenic to a disastrous extent when injected into the blood-vessels of living animals. Again, Kihlmann's brilliant research on *Melanospora*, embodied in an earlier portion of this essay, brings to light an extraordinary case of parasitism and adaptation.

Secondly, we really know very little of the mode of life of many Fungi in their earlier stages; we assume, rather than know, that many forms of *Pyrenomycetes*, for instance, are saprophytes. However, less is to be gained by dwelling upon these doubtful matters than by courting criticism of the main point at issue.

I may say, in conclusion, that it was during the study of the parasitic fungus of the Coffee disease (*Hemileia vastatrix*) in Ceylon that I was first led to speculate on the enormous amount of energy displayed by an organism which shows not the remotest satisfactory trace of sexuality, but which reproduces itself through many generations exclusively by means of asexual spores. That this energy of reproduction is derived from the Coffee tree there can be no doubt, and that it is at the cost of the reproduction of the host is sadly evident; the clear inference from the fact that the Coffee leaf supplies substance for the reproduction, &c., of a Fungus at the expense of its own fruit, is that the Fungus takes matters which are very rich in energy, so rich, indeed, that the Fungus is not neces-

<sup>1</sup> Cohn's 'Beiträge,' B. iii, H. iii, pp. 397 ff.

sitated to sort these substances in special reproductive organs, and to secrete sexual elements, one of which would then reinvigorate the other, but may employ them forthwith for the purposes of its own relatively simpler existence and reproduction.

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# On Procalistes, a Young Cephalopod with Pedunculate Eyes, taken by the "Challenger" Expedition.

By

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MR. JOHN MURRAY, the director of the "Challenger" publications has kindly placed in my hands for examination three specimens of a very young Cephalopod—mounted for microscopic study—together with a drawing and notes by the late Dr. R. von Willemoes Suhm relating thereto.

Dr. Suhm's drawing is reproduced in the woodcut, fig. 1. The following note from his journal refers to these specimens :

"16th June, 1874.—Among the surface gatherings there is a transparent and very interesting Pteropod, with large eyes on the tentacles and without any 'ptera' or foot. Having obtained three more or less damaged specimens from which I could not complete its anatomy, I shall have to defer giving a proper account of it. The animal belongs to the Clionidæ, and is probably allied to Pelagia, Quoy and Gaimard."

With Suhm's drawing of the animal are the following notes :

"Clionid Pteropod: June 16th—18th, 1874. In the warm East Australian current coming from the north (surface temperature 18° C.), together with Calcarella on the voyage from Sydney to Wellington, lat. 34° 50' S., long. 155° 28' E. In all only three specimens, of which the largest alone showed the eyes well. It measured 13 mm. long; tentacles 6—7 mm. long; eye-peduncles 2 mm. long. Neither of the smaller specimens showed anything new. Tentacles with suckers, of

which one is strongly magnified below (woodcut, fig. 1, b). Mouth with six suckers, two teeth, and radula; the latter, as far as I could make it out without injury to the animal, is drawn below to the right hand side (woodcut, fig. 1, c). The mouth leads into an œsophagus; this into a muscular stomach, in the muscular wall of which is a unicellular gland à la nematode. Sharply defined intestine which I could not follow out to the anus on the process to the right (woodcut, fig. 1, f). Ganglion superius sends out the nerves to the eyes; between it and the ganglion inferius are the two otolithic vesicles. On the right side the generative gland is seen with reddish oil specks, and in the corner black pigment; to the left is a cellular body, probably an excretory organ. Subsequently it seemed to me as though there were a calamus in the hindmost portion of the animal; this must, however, have been a mistake. Heart not seen."

It is obvious from the above notes that Suhm had not completed his examination of this interesting organism. The three specimens, of which only two are in such a state as to be useful for examination, have been carefully studied by me, and from these and the information afforded by Suhm, I have constructed a second figure (woodcut, fig. 2), which is placed by the side of Suhm's original drawing. Suhm's drawing being made from fresh specimens affords evidence of various interesting details, and I have thought it right therefore to reproduce it intact. The preserved specimens studied by me are also much older than that drawn by Suhm, which is apparently the one which has completely decomposed. This specimen is half the length of the other two, and whilst it, as shown in Suhm's drawing, possessed suckers both on the long arms and near the mouth, no suckers at all are present in the larger examples. They may possibly have been rubbed off by rough usage of the specimens, but I incline to believe that they are naturally absent in the later stage, though present in the youngest stage as drawn by Suhm. Probably owing to its firm contraction in the living condition, the mantle-flap escaped altogether the observation of Suhm, and this led him to the notion that the animal



before us was a Gymnosomatous Pteropod. That notion was further encouraged by the existence of only two arm-like processes of the forefoot, bearing suckers, these having, as must be at once admitted, a strong resemblance to the sucker-bearing arms of the Gymnosomatous Pteropod, *Pneumodermon*. When once the mantle-flap and the subpallial chamber are overlooked, it is natural to interpret the conical process marked *f* in figs. 1 and 2 as the anus, and to conclude that the supposed Pteropod has no representative of the mesopodium or "ptera." In reality, however, the little creature is not a Pteropod, but one of the Siphonopoda (the group to which the term Cephalopoda is usually restricted). It is not gymnosomatous, but as shown in fig. 2, it has the usual mantle-flap and subpallial chamber characteristic of the cuttle-fishes. The supposed anal cone (figs. 1 and 2, *f*) is in reality the funnel or siphon, and the true anus is placed within the subpallial chamber near the spiral mass of pigment noted by Suhm (figs. 1 and 2, *g*).

The rolling up of the two lateral growths of the mesopodium to form a funnel or siphon is the absolute and distinctive race-mark of the Siphonopoda or Cephalopoda *sensu restricto*. There can therefore be no further doubt about the affinities of Suhm's organism. At the same time I may point out a few additional characteristics which it presents, and are only to be found among the cuttle-fishes.

(1) Near the anus (*g*) is a spiral mass of black pigment. This is the secretion of the ink-sac seen through the walls of that sac. The spiral form of its lumen is characteristic. The ink-sac is distinctively characteristic of the Dibranchiata.

(2) A number of chromatophor-cells, exactly resembling those of young *Loligo*, are scattered over the surface of the body in the integument (fig. 2, *h*). Only Dibranchiata are known to have chromatophors of this particular form and appearance.

(3) There is (as Suhm observed, but could not persuade himself to believe) a very delicate (probably horny) "pen" sunk beneath the integument of the antero-dorsal surface (see woodcut, fig. 2, *i*). Such a pen exists only in the Dibranchiate Siphonopoda.

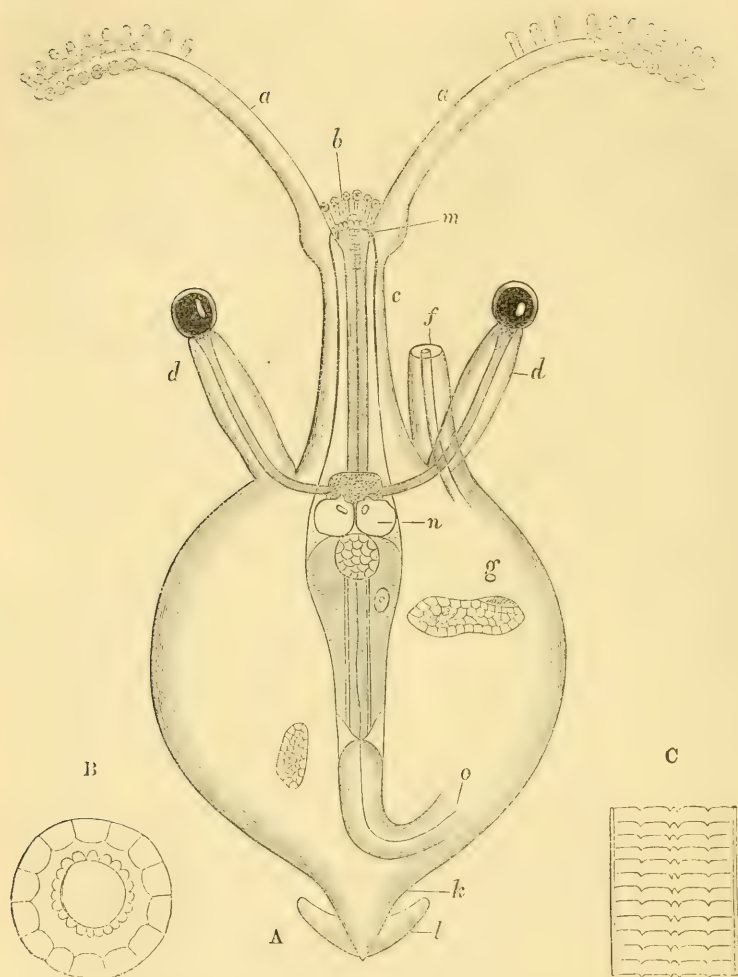


FIG. 1.—A. Youngest specimen of *Procalistes Suhmii*, gen. et sp. nov. Drawn by R. von Willemoes-Suhm from a living specimen. Magnified 25 diameters. *a*. The long “arms” or processes of the fore-foot. *b*. The six small suckers, representing the eight short processes of the fore-foot of a typical Decapod. *c*. The elongated neck. *d*. The pedunculated eyes. *f*. The funnel or siphon. *g*. The anal process seen through the transparent mantle. *h*. The median posterior process of the body. *i*. The lateral fins attached to the same. *j*. The buccal apparatus. *k*. The oto-cysts. *l*. The intestine. B. One of the suckers of the long arms, more highly magnified. C. A portion of the lingual ribbon, more highly magnified.

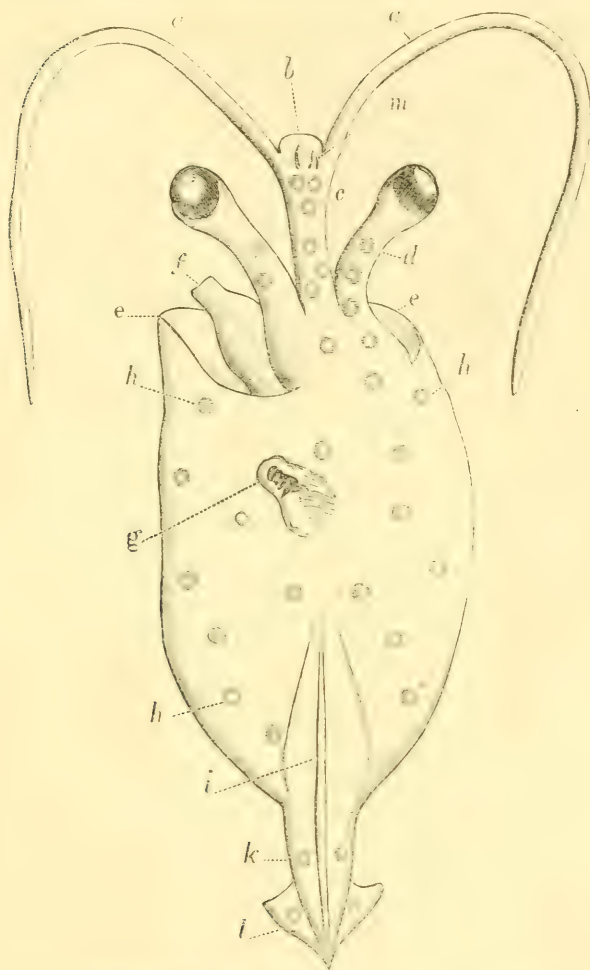


FIG. 2.—A somewhat older specimen of *Procalistes Suhmii*. Drawn by E. Ray Lankester from a specimen mounted on a glass slide in balsam by R. von Suhm. Magnified 20 diameters. *a*. The long "arms" or processes of the fore-foot. *b*. The smooth buccal margin devoid of processes. *c*. The elongated neck. *d*. The pedunculated eyes. *e*. The edge of the mantle flap, separated from its attachment to the head and funnel by pressure. *f*. The funnel or siphon. *g*. The anal process seen through the transparent mantle, and showing a spiral band of black pigment lying in the ink-bag. *h*. Chromatophores. *i*. The pen. *k*. The median posterior process of the body. *l*. The lateral fins attached to the same. *m*. The two horny beaks of the buccal apparatus.

(4) The sharp horny beaks placed at the entrance to the mouth (fig. 2, *m*) are unlike the buccal armature of any mollusc excepting the true cuttle-fishes.

This Clionid-like form is then without doubt a very young condition of a Dibranchiate Siphonopod. In some details it presents important resemblance to the genus *Cranchia*.

The genus *Cranchia* was founded by Leach in 1817; three species are described from the Atlantic Ocean. I take the following characters from 'Bronn's Thierreich.' The body is globular, with terminal paired fins carried on a special prolongation of the body. The mantle is attached to the head by a nuchal band, and is fused on each side to the base of the funnel. (A similar disposition in his young Cephalopod accounts for Suhm's not having detected the free edge of the mantle-flap in the fresh specimens observed by him. Under pressure, when mounted with a cover-glass for the microscope, the mantle has become detached from the base of the funnel, as represented in my drawing, fig. 2, which must therefore be regarded as representing the animal in an artificial condition.) The head is small, with large eyes; the cornea presents only a small slit. The two prehensile arms are long; the smaller arms, eight in number, very short. There are two rows of suckers to the arms. The funnel is long; it is devoid of attachment to the head, and without internal valve. The pen extends along the whole length of the back (antero-dorsal surface), and is thin, soft, small, and pointed at each end.

This description applies in most respects to the young Cephalopod now in question. The differences and peculiarities presented by the "Challenger" specimens are, on the one hand, such as might possibly occur in the young form as compared with the adult; on the other hand they are more probably due to the fact that we have before us a new genus allied to *Cranchia*.

The important peculiarities presented by Suhm's young Cephalopod are:

- (1) The pedunculation of the eyes.



(2) The exceedingly rudimentary character of the shorter arms or perioral processes of the forefoot in the youngest stage observed (fig. 1, *b*), and their total absence as well as the disappearance of the suckers of the long arms in the older specimens.

The elevation of the eyes on stalks relatively so long and so well-marked as in the present instance, is not, I believe, known in any other Siphonopod. Possibly it is only a transient arrangement — disappearing as growth proceeds; but such an elevation of the eyes is not presented by the young of *Sepia*, *Loligo*, *Octopus*, or *Argonauta*, which are the only members of the group whose young forms are certainly known.

The rudimentary character of the perioral arms is very remarkable. Suhm describes them simply as “six suckers.” In the preserved specimens (which it is necessary to point out are in a very poor condition) there is no trace of any perioral suckers or processes. It is important to notice that in Owen’s figure of *Cranchia scabra* (reproduced in Bronn) eight small perioral lobes or arms bearing suckers are figured, of which six are much larger than the other two. It might be possible to regard Suhm’s drawing as indicating a young condition of these six perioral lobes, but the fact that they disappear instead of growing bigger in the older specimens, necessitates a different conclusion. Suhm’s Cephalopod must be placed in a new genus which stands alone in the fact that its suckers and also its perioral foot-lobes, excepting the long pair, are aborted.

For this genus I propose the name *PROCALISTES* (in allusion to H.M.S. “Challenger”), whilst the species can best be named after its discoverer, *P. Suhmii*.

The genus may be defined thus:

— Similar to *Cranchia*, excepting that the eyes are pedunculate, that the shorter perioral arms are aborted, and that the longer (so-called prehensile) arms are devoid of suckers. In the youngest stage observed there are two rows of suckers on the long arms, and six isolated and pedunculated suckers sur-

rounding the mouth, which appear to represent the shorter arms of other Cephalopods.—

I cannot conclude this notice without drawing attention to the correctness of Suhm's recognition of a general resemblance between his young *Procalistes* and such a Pteropod as *Pneumodermon*. The reduction of the forefoot in the former to the condition of two long sucker-bearing arms and a minute set of perioral sucker-bearing processes, finds its parallel—its “homoplast,” if I may use a term introduced by me some years ago—in the condition of the same parts in *Clione* and *Pneumodermon*.

Lastly, is there not some resemblance to the condition of the *Belemnitidæ* in the marked projection of the terminal region of the body to which the lateral fins are attached, and in which the pen (in these young specimens at any rate) is most strongly developed, as also occurs in the living genus *Ancistrocheirus*?

Notes on Echinoderm Morphology, No. VIII.  
On some Points in the Anatomy of Larval  
Comatulæ.

By

**P. Herbert Carpenter, D.Sc.,**

Assistant Master at Eton College.

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With a Woodcut.

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WHILE preparing the morphological section of my report on the "Challenger" Crinoids, I have continually felt the want of some knowledge of the organogeny of the Crinoid-type during the later larval stages. Götte's admirable observations on the "Cystidean phase," and the early Pentacrinoïd, have told us much about the development of the water-vascular ring; while Ludwig's researches have thrown considerable light on the relations of the primary water-pore and water-tube.<sup>1</sup> But in neither case were the larvæ studied sufficiently advanced to afford any results as regards the earliest condition of the chambered organ, and of the puzzling glandular structure connected with it.

These points, however, were just those about which I desired information; and I therefore took steps to obtain a supply of larvæ for the purpose. A. R. Hunt, Esq., M.A., F.G.S., of Torquay, was good enough to supply me with a considerable number which he had dredged in Torbay; and several more were sent me from the zoological station at Naples, to the

<sup>1</sup> "Über den primären Steinkanal der Crinoideen, nebst vergleichend anatomisch Bemerkungen über die Echinodermen überhaupt," 'Morph. Stud. an Echinod.,' Bd. ii, pp. 34-45.

officers of which as well as to Mr. Hunt, my best thanks are due for their efforts on my behalf.

I had at first simply intended to incorporate my observations in the general discussion of the vascular system in the "Challenger" report. But the subject has also been taken up by Professor Perrier, whose views respecting the vascular system of the Crinoids are entirely different from those of Ludwig and myself; and some of the conclusions at which he has arrived are of such a startling and bewildering nature that I am anxious to discuss them at once, so as to clear the way for the general summary of the question which will appear in the report.

Perrier's observations, like so many of his previous ones, are recorded in a brief note of three pages in the '*Comptes rendus*,'<sup>1</sup> and hardly any reference is made to those results of other workers, with which his own conclusions are in direct conflict. In fact, as will be seen immediately, some of his most recent statements are absolutely irreconcilable with those contained in his last note;<sup>2</sup> while others are made in such a guarded manner that it is difficult to understand whether he really means all that his words imply.

Let us consider, first of all, what he says about the primary water-tube and water-pore in the cystid phase, the relations of which have been so well described and figured by Ludwig.<sup>3</sup> According to the German author, the primary water-tube depending from the water-vascular ring opens below into a section of the body-cavity that is cut off from the rest by a band of connective tissue; while the primary water-pore which pierces the oral plate, opens into the same space. The course of both water-pore and water-tube is somewhat curved, but the two organs are not so absolutely continuous as to form one tube. Perrier, however, tells us that this single curved water-

<sup>1</sup> "Sur le développement des Comatules," '*Comptes rendus*,' tome xcviii, No. 7, February 18th, 1884, pp. 444—446.

<sup>2</sup> "Sur l'organisation des Crinoïdes," *ibid.*, t. xcvii, No. 3, July 16th, 1883, pp. 187—189.

<sup>3</sup> *Op. cit.*, p. 39, Taf. xii.



tube of the cystid phase "s'ouvre à l'extérieur par un pore situé sur la paroi du corps." He does not refer at all to Ludwig's careful description of the independence of the inner ends of the water-tube and water-pore respectively; and as he gives no figures in illustration of his statement, I am not inclined to believe it, especially as it would be totally inconsistent with our knowledge of the structure of these organs in the later stages of development, and in the adult Crinoid, as Perrier's own observations show.

Towards the end of the next or pentacrinoid stage there are five tubes and five pores, one in each interradius; and Perrier admits that the inner ends of these "tubes hydrophores" appear in his sections "se terminer par une extrémité libre dans la cavité générale, mais nous avons des raisons de penser que ces tubes sont normalement en continuité," with the inner ends of the fine funnel-shaped water-pores. He does not state these reasons, however, but goes on to say that in the latest pentacrinoid condition, just when the young animal is about to detach itself, "les tubes hydrophores se sont considérablement multipliés, mais on observe les memes rapports entre eux et les canaux qui traversent la paroi du corps pour s'ouvrir à l'extérieur."

I have not made many observations regarding this point, as I considered it to have been settled by Ludwig; but from what I have seen I am fully prepared to support his view of the want of connection between the primary water-tube and water-pore; while there is every reason to believe that the condition of the Pentacrinoid is represented permanently by that of Rhizocrinus. This type has only one tube and one pore in each interradius, and they are not directly continuous, but communicate with one another through the intervention of the body-cavity.

Perrier's last statement respecting the multiple water-tubes of the late Pentacrinoid and young Comatula is a very cautious one. It reads as if he meant to imply that the

<sup>1</sup> I have only found one pore in larvæ with a completely formed chambered organ, while individuals with five cirrus-stumps have only two.

numerous pores in the interpalmar areas of the disc are in direct continuity with the tubes depending from the water-vascular ring in the lip, just as he asserts to be the case in younger larvæ. If this be true, then he has discovered a fact which has escaped the notice of Grimm, Greeff, Teuscher, Ludwig, my father, and myself; and the sooner he gives us a straightforward description and figure of it, the better. If, however, this is not his meaning, one would like to know what relation between the water-pores and water-tubes he really does intend to describe. He states in his concluding résumé that “jusqu'à cet âge les pores qui font communiquer la cavité générale avec l'extérieur peuvent être considérés comme les orifices de tubes hydrophores avec lesquels ils sont liés tout à fois par leur nombre et leur position.” It is difficult to reconcile this with his previous assertion respecting the absolute continuity of the primary water-pore and water-tube without the intervention of the body-cavity.

The most startling statements which he makes, however, are those relating to the homologies of the problematical structure which I have called the plexiform gland. It has been designated by a variety of names, as is shown in the following list:<sup>1</sup>

Perrier stated in his note of last July, and rightly, as I believe, that “L'organe dorsal des Crinoïdes a la même structure que le prétendu cœur des autres Échinodermes.” But he now says that while the water-pores and water-tubes of a Crinoid “ne correspondent nullement au canal du sable (water-tube or stone-canal) des autres Échinodermes; ce canal du sable paraît au contraire représenté par l'organe axial des Comatules, qui possède tout à fois la structure du canal du

<sup>1</sup> W. B. Carpenter, 1875, ‘Axial prolongation.’

Greeff, 1876, ‘Drusenartigé dorsoventral Gefässaxe.’

Ludwig, 1877, ‘Dorsales Organ;’ 1880, ‘Centralgeflecht.’

P. H. Carpenter, 1881, ‘Central plexus.’

Perrier, 1883, ‘L'organe dorsal,’ ‘Le glande ovoïde,’ ‘Le corps ovoïde.’

P. H. Carpenter, 1883, ‘Plexiform gland.’

Perrier, 1884, ‘L'organe ovoïde,’ ‘L'organe axial.’

sable des Astéries et la position de l'organe de même nom chez les Oursins."

Let us first endeavour to determine the reasons which have induced Perrier to compare it, not with the ovoid gland of Starfishes and Urchins, with which he has previously told us that it corresponds in structure, but with the stone-canal of these types. The stone-canal of a Starfish or Urchin is generally supposed to be lined by a single layer of columnar epithelium, parts of which at any rate are ciliated like that within the water-tubes of a Crinoid, and totally different from the close cellular tissue forming the axial organ.

Perrier describes the axial organ in the cystid phase of *Comatula* as "un corps ovoïde, dont les grandes cellules sont toujours, sur les coupes, disposées en deux rangées contiguës, de sorte que le corps ovoïde est plein."

In the pentacrinoid stage it is said to have "l'aspect d'un double canal dont les deux parties semblent s'ouvrir dans le pharynx." It has almost the same histological structure as in the preceding phase; while in the mature larva there is the same cellular structure, "mais ses parois se recourbent intérieurement en lames enroulées qui rappellent d'assez près les dispositions du canal du sable des étoiles de mer."

As Professor Perrier has not yet described the stone-canal of a Starfish, it is difficult to know exactly what grounds he has for his opinions. The mere fact that the walls of the axial organ in a young *Comatula* are plicated like those of the stone-canal in Starfishes, scarcely seems to me a sufficient foundation for the conclusion that a part of the water-vascular system in the latter group is represented in the Crinoid by a structure which is generally considered (outside France) as connected with the blood-vascular apparatus.

It is curious, too, that this last point should receive support from Perrier's most recent observations. In his previous note he stated that "Le corps ovoïde s'implante, chez la Comatule adulte, sur l'un des planchers horizontaux de l'organe cloisonné." Now, however, he tells us that "cet organe se

termine inférieurement en un tube conique qui pénètre en s'amincissant toujours, dans l'axe de l'organe cloisonné."

This connection was described by Ludwig seven years ago in *Antedon* and *Rhizocrinus*; and I have since stated that I had been able to confirm his observations in both these types, and had extended them to *Actinometra*, *Pentacrinus*, and *Bathycrinus*. One would think, however, from what Perrier says, that he was describing an entirely novel observation. At any rate, I am glad to find him admitting that this axial organ is the upward continuation through the chambered organ of a structure which runs down the larval stem as a "cordon central" with five "cordons périphériques" around it; and that the latter enlarge at the top of the stem to form the five cavities of the chambered organ. Considering the minute size of these cordons in the stem of the *Pentacrinoid*, one could hardly expect them to possess a lumen. But the case is very different in the stalked Crinoids. Ludwig long ago described and figured the six vessels, one central and five peripheral, in the stem of *Rhizocrinus*; and I have mentioned their pre-

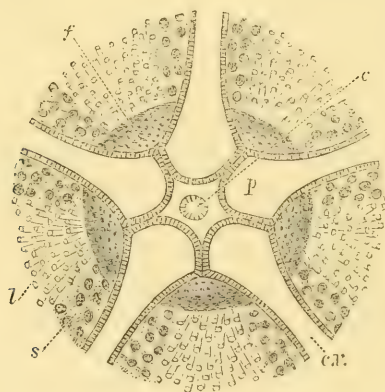


FIG. 1.—The central part of a horizontal section through a nodal joint in the stem of *Pentacrinus*.  $\times 90$ . *c*. Central vessel. *p*. Peripheral vessels, expanded into miniature "chambers." *c.v.* The cirrus-vessels proceeding from these chambers. *f*. Fibrillar sheath of the vascular axis. *l*. The interradial tendons of the stem. *s*. Organic basis of the skeleton.



sence in *Bathycrinus* and *Pentacrinus*, stating at the same time that in the latter type the cirrus-vessels are given off from enlargements of the peripheral vessels within the nodal joints. This is well shown in fig. 1. Except for the presence of the stem-ligaments (*l*), this figure would very well represent a horizontal section through the lower part of the chambered organ of a *Pentacrinoid* with five cirri. The vessels which proceed to these cirri start, as might be expected, from the peripheral chambers, and not from the central vessel; and the five first cirri are radial in position, just like the cirri on the stem of *Pentacrinus*. I wish particularly to insist upon this point; for the primary cirri have sometimes been described as interradial, owing to the one of them being opposite to the anal plate which at first separates two of the radials. This condition, however, is only transitory in *Ant. rosacea*, all the radials ultimately coming into close lateral union, with a cirrus-socket on the centro-dorsal immediately beneath the middle line of each. A good figure showing this point is given by Dr. Carpenter.<sup>1</sup>

I have examined a quantity of larvæ from Naples, Torbay, and Arran, all of which have the cirrus-stumps overlying the interbasal sutures, and therefore radial in position. Sars has noticed the same feature in *Ant. dentata*, and has illustrated it by an excellent figure;<sup>2</sup> and I can confirm his observations for the larvæ, both of this species and of two other *Antedons*, one from the "Porcupine," and the other from the "Challenger" dredgings. In all these three larvæ the cirri do not appear till the radials have met laterally and the anal plate has been lifted altogether out of the calyx, so that there is no possibility of mistaking their radial position.

Now let us turn to Perrier's account of their development. He tells us in the first place that the arms are formed by "bourgeons cellulaires partant du sommet" of the cavities of the chambered organ, which unite with similar "bourgeons" from the water-vascular ring. The chambers, however, have

<sup>1</sup> 'Phil. Trans.,' 1866, Pl. xli, fig. 6.

<sup>2</sup> 'Crinoïdes Vivants., pp. 53—66, Taf. viii, fig. 11.

nothing to do with the arms, as Perrier appears to think, except that both are radially situated. No part of the chambers extends into the arms, the axial cords of which are derived from trunks that start from the interradial angles of the chambered organ. This, however, is a minor point. But Perrier continues to describe how at the level of the chambered organ "du cordon pédonculaire central, on voit, chez les individus dont les bras sont encore peu développés, naître des bourgeons claviformes alternes avec ceux qui, des bras" (sic). These are the rudiments of the cirri. "Les cirrhes n'ont donc pas de véritable homologie avec les bras; ils naissent du cordon central du pédoncule; les bras, des cinq cordons périphériques." In reply to this astonishing statement I would simply remark: (1) The arm-rudiments are in no way derived from the peripheral vessels (cordons) of the stem, which expand above into the cavities of the chambered organ; (2) The cirri are radial, like the arms, and do not alternate with them; (3) How is the connection effected between the cirri and the "cordon central" of the stem? The latter is closely surrounded by the "cordons périphériques," which represent the five outer vessels in the stem of *Pentacrinus* (fig. 1), and no extensions proceed outwards from it between the radial (peripheral) vessels, as Perrier's description demands.

He asserts that the axial organ (= stone-canal of a Starfish!), which he admits to be the upward continuation of the "cordon central," is "évidemment en rapport avec la nutrition des cirrhes." But unless he will further admit that the cirrus-vessels are radial and derived from the cavities of the chambered organ, and also that these cavities themselves pass upwards into the axial organ, I cannot accept his statement as at all consistent with the observations of Ludwig and myself.

He has likewise told us that the upper end of this axial organ in the *Pentacrinoid* seems to open into the pharynx; but he admits (as well he may) that further observations will be necessary before he can satisfy himself upon this point. He again ignores Dr. Carpenter's description of its subdivision

"into diverging branches, of which one passes to each ray."<sup>1</sup> Some of these branches may, I believe, be readily made out in optical sections of Pentacrinoids soon after the appearance of the cirri; but in most of the larvæ which I have examined by this or by the section method these branches have not yet appeared, the axial organ ending close against the pharynx, though in no way connected with it.

The ramifying tubules which depend from the blood-vascular ring of the adult, and form the labial plexus that unites this ring and the axial organ (plexiform gland), do not seem to develop until some time after the appearance of the cirri, for I have seen no trace of them in any Pentacrinoid. The inter-visceral vessels and those forming the genital plexus are also late in development. But the latter seem to have been observed by Perrier himself in the recently liberated Comatula. For he says that within the meshes of the connective-tissue network which occupies the body-cavity, there "courent un petit nombre de cordons cellulaires pleins qui se rendent manifestement aux bras." Are not these a further development of the branches from the axial organ, of which one passes to each ray, as described by Dr. Carpenter?

In conclusion, I would reiterate the hope which I expressed in a previous note, that Professor Perrier will soon publish a complete account of his views respecting the vascular system of the Crinoids, both larval and adult; and that he will illustrate it by plenty of those figures which he knows so well how to draw. The evidence which I have for the various statements that have appeared in this journal will be given in some eight or ten plates of the "Challenger" report.

<sup>1</sup> 'Proc. Roy. Soc.,' vol. xxiv, p. 221. I can confirm this statement from examination of the dissected larva on which it was founded.

Some Account of *Polystigma rubrum*, Pers.,  
based upon the Recent Investigations of Dr.  
A. B. Frank,<sup>1</sup> and C. Fisch.<sup>2</sup>

By

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Society.

*Polystigma rubrum*, Pers. in 'Mougeot and Nestler's Stirp. Cr. Vog.,' No. 270 (1812)—Decand., 'Fl. Fr.,' vi, 164; 'Mém. du Mus.,' iii, 337, pl. xiv, fig. 7 (1817)—Grev., 'Fl. Edin.,' p. 365; and 'Scot. Cr. Fl.,' ii, pl. 120 (1824)—Fresen., 'Beitr. zur Myk.,' i, 34 (1850)—Rabenh., 'Herb. Mycol.,' ed. 2, New Ser., No. 580 (1857)—Tulasne, 'Sel. Fung. Carp.,' ii, 76—8, pl. viii, figs. 10—20 (1863)—Fuckel, 'Symb. Myc.,' p. 222 (1869); 'Nach.,' ii, 40 (1873); ditto, iii, 25 (1875); exs. Nos. 1003 (Spermog.), 2664 (Asci)—Cooke, 'Hand. Br. Fung.,' p. 803 (1871); exs. i, 182; ii, 577—Saccardo, 'Mich.,' ii, 282 (1878); v, 551 (1879); vi, 74 (1880)—Roumeg., 'Fung. sel. Gal.,' exs. No. 275—Stevenson, 'Myc. Scot.,' p. 364 (1879)—Frank, 'Krankh. der Pflanz.,' p. 633-4 (1880)—Rabenh., 'Deutsch. Kr. Fl.,' i, 166 (1844).

*Xyloma* (?) *rubrum*, Pers., 'Obs. Myc.,' ii, 101 (1799); *X. rubrum*, 'Syn. Meth.,' p. 105 (1801)—Alb. et Schwein., p. 64 (1805)—Hooker, 'Fl. Scot.,' ii, 9—Purton, 'Mid. Fl.,' iii, 316, pl. xxxiii (1821)—Nocca et Balbis, 'Fl. Tic.,' ii, 808,

<sup>1</sup> "Ueber einige neue und weniger bekannte Pflanzenkrankheiten," 'Berichte der Deutschen Bot. Gesellschaft,' i, p. 58—62 (1883).

<sup>2</sup> "Beiträge zur Entwicklungsgeschichte einiger Ascomyceten," 'Botanische Zeitung,' Nos. 49—51 (1882).



pl. xxv, fig. 1, sec. Saccard. (1821)—Schum., 'Flor. Saell.,' ii, 179 (1803).

*Sphæria rubra*, Fries, 'Obs. Myc.,' i, 172 (1815)—Kunz. et Schm., 'Myk.,' Heft ii, 24 (1823)—Wallroth, 'Flor. Cr.,' ii, 847 (1833)—Link, 'Handb.,' iii, 365 (1833).

*Sphæria hyetospilus*, Martius (pro parte), 'Fl. Crypt. Erlang.,' p. 478 (1817)—Fr. Nees, 'Nov. Act. Ac. L.-C. Nat. Cur.,' ix, 253, pl. vi, fig. 21 (1818).

*Dothidea rubra*, Fries, 'Syst. Myc.,' ii, 553 (1823); 'Sum. Veg. Sc.,' p. 387 (1849); exs. No. 191—Berk., 'Eng. Fl.,' p. 286 (1836); 'Outlines Brit. Fung.,' p. 391 (1860).

*Ascochytae* sp., Libert, 'An. Sc. Nat.,' Ser. 2, vii, 124 (1837).

*Septoria rubra*, Desmaz., 'Pl. Crypt. Gall.,' Ed. 2, xv, No. 734 (1843); 'An. Sc. Nat.,' Ser. 2, xix, 342 (1843), var. *β. amygdali*.

*Libertella* sp. (*rubra*), Bonord., 'Handb. Mykol.,' p. 55, (1851).

Recorded from England, Wales, Scotland, France, Germany, Italy, America, &c.

This fungus-parasite on species of *Prunus* does not appear to have been so injurious in this country as on the Continent, where it has in certain places, as at Berlin, committed great ravages in fruit orchards. It usually makes its appearance a little before midsummer as yellow, afterwards red, spots on the leaves of *Prunus domestica*, *P. spinosa*, *P. insititia*, and has also been recorded (at least, in the spermogone stage) on *Amygdalus*. These spots, which are more or less orbicular, and often confluent, from  $\frac{1}{8}$ th to  $\frac{3}{8}$ ths of an inch in diameter, are developed on both surfaces of the leaf, but more particularly on the lower; they consist of the abundant mycelium of the fungus closely compacted with the hypertrophied mesophyll of the leaf, which thereby becomes somewhat thickened, and of a fleshy consistence. The chlorophyll of the attacked cells is destroyed, and the mixed tissue or stroma assumes the characteristic fiery hue, which reminds one, as all authors who touch upon the subject have noticed, of the early stage of an

*Æcidium*. The epidermis of the leaf remains uninjured, as also the fibro-vascular bundles which traverse the affected part.

The elevated and convex surface of the stroma, usually on the under side of the leaf, is covered towards the end of June with numerous minute scarcely perceptible darker dots, which are the openings or ostiola of the spermogones. These spermogones originate within the stroma in dense coils of interwoven hyphæ, which at length form a hollow ball of deep-red pseudo-parenchymatous tissue, .1 mm. in diameter, which penetrates the epidermis by its obtusely conical apex, terminating in a pore, while the inner surface of its wall is clothed with a dense layer of linear, straight, simple basidia, from which the spermatia are abstricted. The latter are very slender, filiform, attenuated and uncinatæ above, about 30 mk. long; as they accumulate, they are forced out of the ostiolum, and being involved, as is usual in such cases, in a gelatinous mucus, are heaped in a globule round the apex. These spermatia continue to be developed until the middle of October.

So much was known, in the main, to Tulasne; he was also acquainted with the subsequent development of perithecia and the ripening of the ascospores in the succeeding spring. But he believed that the perithecia did not appear upon the living leaves, being developed only after they had fallen from the tree and lay rotting on the ground; in fact, that the spermogones had ceased to be developed before the formation of the perithecia began, and therefore the connection between the two, if any were suspected to exist, could only be indefinitely imagined. It is here that the recent discoveries of Frank and Fisch throw a new light and add another to the cases already known where a true sexual act exists among the Ascomycetes. The investigations of these two authors mutually supplement and confirm each other, and, as they were independently made, give strong reasons for believing in the truth of their conclusions.

The foundations of the future perithecia are laid in the stroma of the fungus, while the leaves are still living and

attached to the tree, about the time when the greater part of the spermogones are completing their development. They are to be found on the same surface on which the spermogones have their mouths, as early as the end of July. They appear at first in a very similar manner, as closely-wound balls of hyphæ, of a deep-red colour, very much smaller than the mature spermogones, but more numerous, and distributed evenly over the stroma without regard to the spermogones. Within each mass one of the hyphæ differentiates itself from the rest, becoming thicker and spirally twisted, and sending out a prolongation which pierces the stroma and projects above its surface as a thick thread, surrounded at its base by more delicate threads. The spiral hypha is called an ascogonium, and the projecting thread a trichogyne. Frank often found the spermatia clustered in heaps round the base of a trichogyne, and occasionally detected one closely united with its apex. In such cases the spermatium was observed to undergo a change; the contents appeared to pass out; it became full of vacuoles, and its outline irregular and by degrees almost imperceptible, while the non-copulated spermatia remained full and regular. Fisch was not able to convince himself of the process of fertilisation, as Frank has done, but if we consider the striking homology between the organs just described as ascogonium and trichogyne, and those known by the same names among the Lichens (for which see the 'Quart. Journ. of Microscop. Science,' xviii, 1878, p. 440, and especially Pl. XX, figs. 1—3); if, moreover, we regard the simultaneity of their formation with the time of greatest development of the spermatia, the enormous numbers of the latter, which render fertilisation almost a certainty, their curious hooked form by which they are enabled to hang on to the trichogyne on the under surface of the leaf, we can, as Frank says, have little doubt of the purpose which these organs serve. In these respects, and in the position of the male and female organs on the lower surface, together with the mucus in which the spermatia are involved, which protects them from being washed away by the rain or dried up prematurely by the sun,

we have a series of adaptations fairly comparable with those which are known to exist in flowering plants.

There is also another point in favour of this conclusion—viz. the decided incapacity of the spermatia for germination. The ascospores, to be afterwards described, germinate readily when mature, but the spermatia will not. This is also known to be a general characteristic of the bodies classed under the latter designation. It is true that Max Cornu ('*Annales des Sciences Naturelles*,' Ser. 6, vol. iii) disputes this, affirming that he has succeeded in causing the spermatia of many *Pyrenomycetes* to germinate. But in the first place it is manifest that the growth which he calls "germination" is something different from what is usually intended by that name; the processes which grew from his spermatia were always short and frequently deformed. In the second place, we must remember that a pollen-grain may be said in a sense to germinate when it sends out a pollen-tube; and the kind of growth which Max Cornu describes is exactly comparable with that which a pollen-grain exhibits in sugar solution. It is obvious that his contention for the non-sexual character of the spermatia on this ground is founded upon a series of errors. Moreover, it must not be forgotten that not all the bodies described by authors as spermatia are necessarily rightly so called.

When the leaves have fallen, the development of the perithecia continues, although more or less delayed by winter, and is completed between February and April. The mature asci are clavate, 65 mk. long, about 10 mk. broad, each containing eight oblong-oval, smooth, one-celled spores, which are straight, obtuse at each end, nearly colourless, 10—13 mk. by 6 mk., and sometimes pseudo-septate in the middle. Paraphyses wanting. Placed in moisture they germinate forthwith, putting out a short tube which forms at the end an oblong swelling; into this, which reaches about the size of the spore, the whole or the greater part of the contents pass, it is then cut off by a septum, and the membrane becomes brown in colour. This swelling is always closely appressed to the surface on which it grows; when several are together they



become longer and grow round one another in dense spiral convolutions. They are designated by Fisch "secondary spores," but Frank considers that they are merely clinging organs, "Haft-organe," being very variable in form and incapable of germination. When they are placed upon the cuticle of a plum leaf they give off sac-like prolongations, which penetrate into the interior of the cells, and from which the mycelium of the stroma is again produced.

The fallen leaves soon rot, except in the parts occupied by the stroma of the Polystigma; these become brown and then blackish, persisting even till the following July, and furnishing up to that time a constant supply of fresh ascospores. As they dry they curl up with the convex surface, on which the openings of the perithecia are situated, directed outwards, and are easily rolled along by the wind. Fresh infections of the host-plants continue to originate up to the end of July. The spores will not germinate in the perithecia, but when discharged germinated in two hours at 18° C., and produced their brown clinging organs in twenty-four hours.

It will thus be seen that probably the whole life-history of *Polystigma rubrum* is now known. The only point which is left in doubt is the mode by which the ascospores are conveyed from the ground to the young plum leaves. Frank had the idea of invoking the aid of insects for this purpose, but, since the spores are small, it would seem that the wind is capable of doing all that is required. The infection of the host-plants therefore takes place from the foliage of the preceding year. But the collection and removal of the old leaves would not avail to prevent a new outbreak if the mycelium hibernates, as that of so many Fungi does, in the woody tissue of the tree. To settle this point Frank investigated carefully the twigs of the affected plants, but could find no traces of mycelium, not even in the petioles of leaves severely attacked by the fungus. In fact, it could be seen that the mycelium ceased at the edges of the stroma. The parasite is therefore annual, and infection takes place only from the ascospores direct. The chief in-

jury which it causes, even in severe attacks, is due to the reduced assimilating power of the foliage which necessarily supervenes when so large a quantity of chlorophyll is destroyed.

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## An Attempt to re-classify the Rotifers.

By

**C. T. Hudson, LL.D.**

FIVE-AND-FORTY years have elapsed since Ehrenberg published his classification of the Rotifera, and his system still holds its ground. The mere statement of the fact is high praise; for what have not classifiers altered, and attempted to alter, during the last half century? Not that his classification has escaped challenge. It was sharply criticised in the '*Histoire naturelle des Zoophytes*' by Dujardin, in 1841; and the author showed by his criticism that he would probably have invented an excellent classification, if he had only had the requisite knowledge. For his arrangement of the Rotifers into great groups was excellent, and he failed in his subdivisions, obviously from lack of personal acquaintance with the creatures he was classifying.

Leydig, also, in his admirable treatise '*Ueber den Bau und die systematische Stellung der Räderthiere*,' in 1854, pointed out some of the obvious faults of Ehrenberg's system; and substituted for it a far inferior one of his own.

Lastly, Dr. Samuel Bartsch, in a pamphlet on '*Die Räderthiere*' in 1870, and again in a larger treatise on the '*Rotatoria Hungariæ*' in 1877, has essayed a new classification, which is, I think, by no means a success.

I propose now to point out, as briefly as may be, what seem to me to be the chief faults in these four systems; and then, availing myself of all that has been already done, to see how far the accumulated observations of the last forty-five years will enable us to arrange the Rotifers in well-marked and fairly natural groups. I am sanguine enough to think that this can be done with a large proportion of them; though there may remain outstanding some genera, that can at the best have only

provisional places assigned to them; owing either to their unusual forms, or to their not having been sufficiently studied.

The great majority of the Rotifers carry on their heads lines or clusters of moving cilia, by means of which they swim and conduct food into their mouths. Ehrenberg's first division into groups is in accordance with the supposed forms of these ciliated curves and clusters; and each group is again divided into Rotifers that have, and into those that have not, a lorica or case.

Lastly, these groups are sub-divided into genera mainly in accordance with the presence or absence of eyes, with their number and with their situation; while in the larger groups the form of the trochal disc and foot, and the number of teeth in the jaws, are also made use of to help in separating the genera.

The result is that the Rotifera are divided into four groups, according to the following plan:

#### CLASS—ROTATORIA.

\* A simple continuous ciliary wreath . . . . . Monotrocha.

\*\* A compound or divided ciliary wreath . . . . . Sorotrocha.

##### \* MONOTROCHA.

(a.) An unbroken-edged ciliary wreath . . . . . Holotrocha.

(b.) A scalloped ciliary wreath . . . . . Schizotrocha.

##### \*\* SOROTROCHA.

(a.) A many-parted ciliary wreath . . . . . Polytrocha.

(b.) A two-parted ciliary wreath . . . . . Zygotrocha.

and as each of these groups is sub-divided into a loricated and an il-loricated family, we have finally an arrangement by which all known Rotifers are made to take their places in one or other of the eight families of the following neat and symmetrical system:

|             |   |              |   |                  |                |
|-------------|---|--------------|---|------------------|----------------|
| MONOTROCHA. | { | Holotrocha   | { | il-loricated . . | Ithydina.      |
|             |   |              |   | loricated . .    | Ceistina.      |
|             | { | Schizotrocha | { | il-loricated . . | Megalotrochæa. |
|             |   |              |   | loricated . .    | Floscularia.   |
| SOROTROCHA. | { | Polytrocha.  | { | il-loricated . . | Hydatinae.     |
|             |   |              |   | loricated . .    | Euchlanidota.  |
|             | { | Zygotrocha   | { | il-loricated . . | Philodinae.    |
|             |   |              |   | loricated . .    | Brachionæa.    |



Nothing could be more precise, or more symmetrical; but these merits—dear as they are to most men, and to all classifiers—have been purchased at the expense of grievous faults.

In the first place, there is not a truly loricated animal at all in the whole of the *Monotrocha*. They are all soft-bodied, flexible Rotifers, and the great majority live in gelatinous tubes secreted from their own skins, and strengthened by the adherence to them of foreign bodies. To give such cases the same name as that chosen for the transparent chitinous carapace of a *Brachionus* (fig. 7) is surely an absurdity.

In the next place, the division *Holotrocha* does not really exist. For rejecting *Icthydium* and *Chætonotus* as not being Rotifers at all, as well as the very doubtful genus *Glenophora*, the remaining three genera, viz. *Æcistes*, *Conochilus*, and *Ptygura* have all gaps in their large ciliary circle precisely as *Melicerta* (fig. 9) has. The gap is easily seen in *Conochilus*, and lies on one side of the antennæ, while the mouth is on the other; and in *Æcistes*, although the gap in the ciliary wreath is small and rather difficult to be made out (unless the animal is fortunately placed), still it is there; and it is on the ant-oral side just as it is in *Melicerta* (fig. 9).

Again it is surely a confusing of very unlike things to speak of the nearly motionless setæ of *Stephanoceros* (fig. 10) and *Floscularia* (fig. 11) (often in the latter stretching to the animal's full length) in the same terms as those applied to the ciliary wreath of *Melicerta* (fig. 9). There is no sort of similarity between them; and nothing but the exigencies of a symmetrical system could have led to such a misuse of names.

Strictly speaking too the term *Monotrocha* is as misleading as any that we have already considered; for nearly all the genera included in this group have not one ciliary wreath, but two, running parallel to each other—one of large cilia, and one of small ones, with the mouth lying between the two.

Nor is this all. The sub-division into genera is made to

FIG. 1.

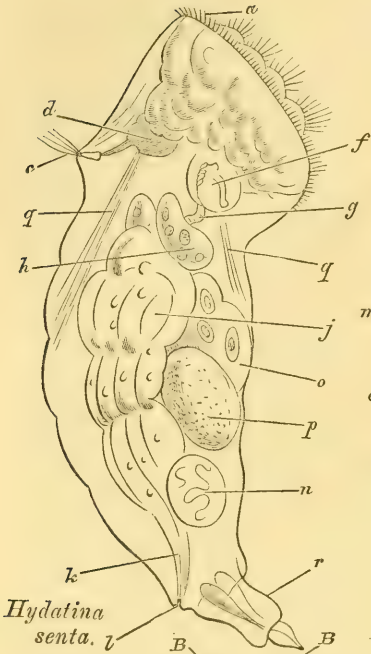


FIG. 2.

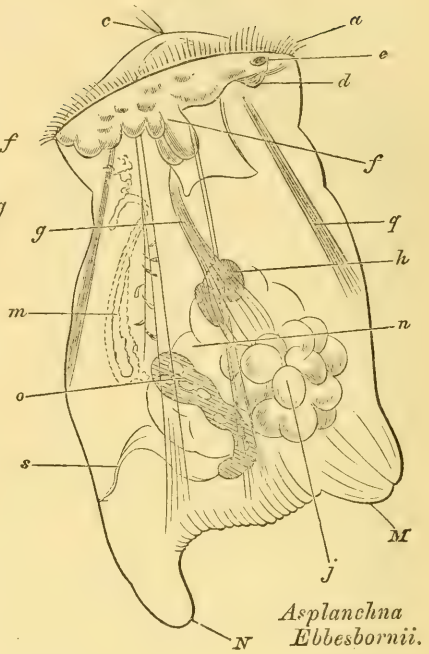


FIG. 3.

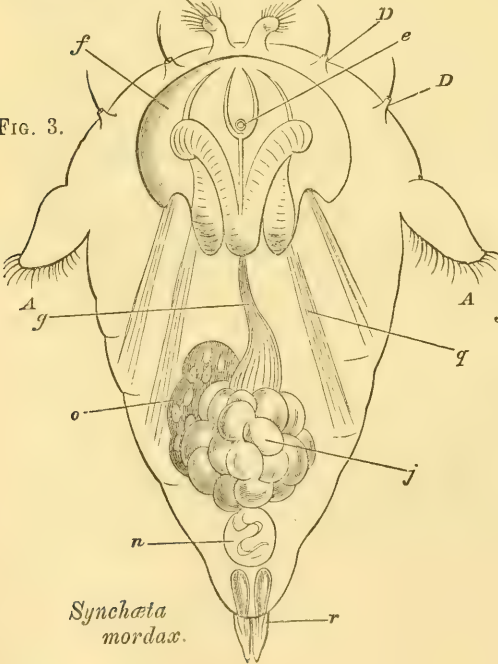
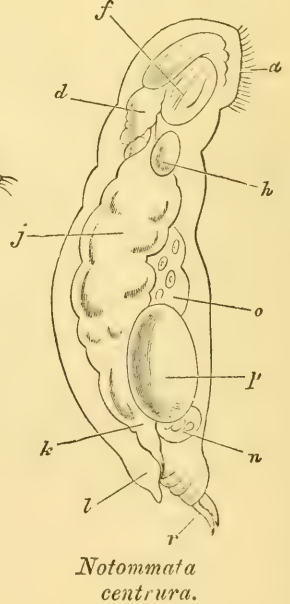


FIG. 4.



## EXPLANATION OF THE WOODCUT ON THE OPPOSITE PAGE.

FIG. 1.—Hydatina.

- a.* Principal ciliary wreath.
- c.* Antenna.
- d.* Cephalic ganglion.
- f.* Mastax.
- g.* Œsophagus.
- h.* Gastric gland.
- j.* Stomach.
- k.* Intestine.
- l.* Anus.
- n.* Contractile vesicle.
- o.* Ovary.
- p.* Ovum.
- q.* Muscle.
- r.* Foot.

FIG. 2.—Asplanchna.

- a.* Principal ciliary wreath.
- c.* Antenna.
- d.* Cephalic ganglion.
- e.* Eye.
- f.* Mastax.
- g.* Œsophagus.
- h.* Gastric gland.
- j.* Stomach.
- m.* Convolted tubes and vibratile tags.
- n.* Contractile vesicle.
- o.* Ovary.
- q.* Muscle.
- s.* Oviduct.
- M.* Dorsal protruberance.
- N.* Ventral protruberance.

FIG. 3.—Synchæta.

- e.* Eye.
- f.* Mastax.
- g.* Œsophagus.
- j.* Stomach.
- n.* Contractile vesicle.
- o.* Ovary.
- q.* Muscle.
- r.* Foot.
- A.* Ciliated side lobes.
- B.* Setigerous prominences.
- D.* Antennæ.

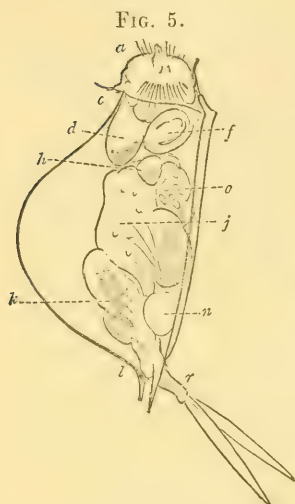
FIG. 4.—Notommata.

- a.* Principal ciliary wreath.
- d.* Cephalic ganglion.
- f.* Mastax.
- h.* Gastric gland.
- j.* Stomach.
- k.* Intestine.
- l.* Anus.
- n.* Contractile vesicle.
- o.* Ovary.
- p.* Ovum.
- r.* Foot.

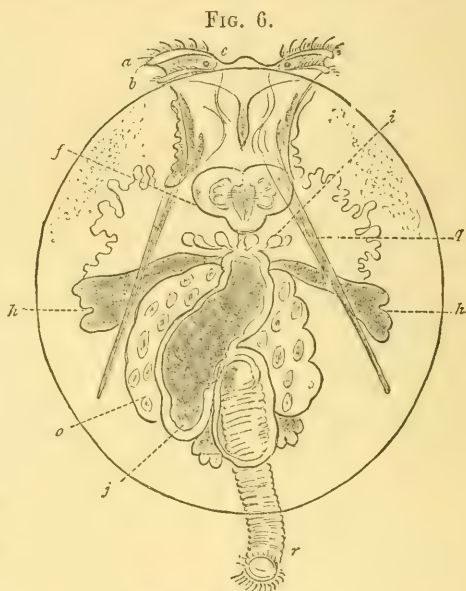
depend on the absence or presence of eyes, and on their number; and here Ehrenburg is not right as to his facts.

For striking out from his fifteen genera of the *Monotrocha*, the three already mentioned with *Cyphonautes*, ten of the remaining eleven genera have two eyes when young.

Though the use of the red eye-spots has on this occasion



*Euchlanis triquetra.*



*Pterodina patina.*

FIG. 5.—*a*. Principal ciliary wreath. *c*. Antenna. *d*. Cephalic ganglion. *f*. Mastax. *h*. Gastric gland. *j*. Stomach. *k*. Intestine. *l*. Anus. *n*. Contractile vesicle. *o*. Ovary. *r*. Foot.

FIG. 6.—*a*. Principal ciliary wreath. *b*. Secondary ditto. *c*. Eyes. *f*. Mastax. *h*. Gastric gland. *i*. Salivary glands. *j*. Stomach. *o*. Ovary. *q*. Muscle. *r*. Foot.

been unfortunate, still I cannot agree with those who object to their being used as generic characteristics, and who doubt of their being eyes at all. In some of the Rotifers, as in *Triarthra longiseta* (fig. 8), *Pedalion mirum* (fig. 12), and *Conochilus volvox*, they are beautiful little diaphanous spheres, resting on plates of ruby pigment, while the splendid eye



of *Microcodon clavus* crowns a rounded ganglion covered with purple segments; and, in all the cases which I have been able to investigate, the eye-spots are either seated on the principal nervous mass, or have nerve-threads passing to them from it.

Unfortunately however it often happens that eyes which are conspicuous in the egg, or in the young, become difficult of detection in the adult. This is the case with *Stephanoceros* (fig. 10) and the *Floscules* (fig. 11); in which genera the eyes of the adults

FIG. 7.

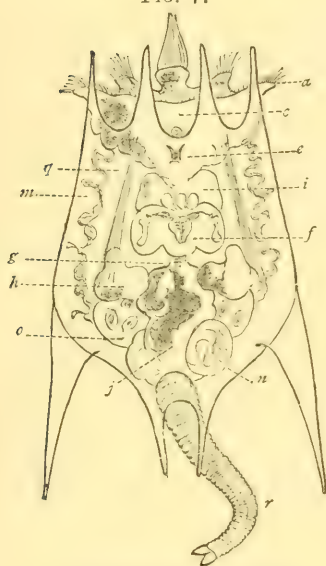
*Brachionus amphiceros.*

FIG. 8.

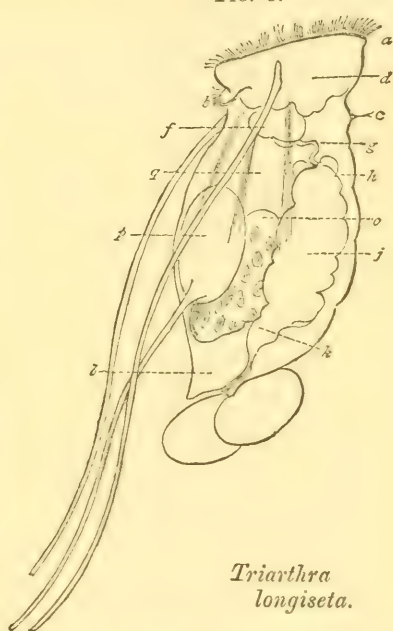
*Triarthra longiseta.*

FIG. 7.—*a*. Principal ciliary wreath. *e*. Eye. *f*. Mastax. *g*. Œsophagus. *h*. Gastric gland, *i*. Salivary gland. *j*. Stomach. *m*. Convoluted tubes. *n*. Contractile vesicle. *o*. Ovary. *q*. Muscle. *r*. Foot.

FIG. 8.—*a*. Principal ciliary wreath. *b*. Secondary ditto. *c*. Antenna. *f*. Mastax. *g*. Œsophagus. *h*. Gastric gland. *j*. Stomach. *k*. Intestine. *l*. Anus. *o*. Ovary. *p*. Ovum.

are best seen by treating them as opaque objects, and throwing a strong light upon them from above. Moreover, there are often red spots on Rotifers which are not eyes at all; so that

on the whole it would seem best to use this characteristic as sparingly as possible, and then only when the structure has been thoroughly made out.

If we now turn to the genera to see how far Ehrenberg's system has brought similar forms together, we find *Cecistes* (which greatly resembles *Melicerta* (fig. 9)) separated from its kinsfolk, and classed with animals some of which are not Rotifers at all; *Conochilus* and *Ptygura* being in the same predicament.

FIG. 9.

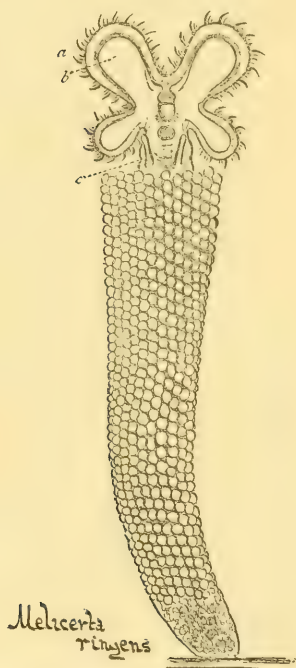


FIG. 10.

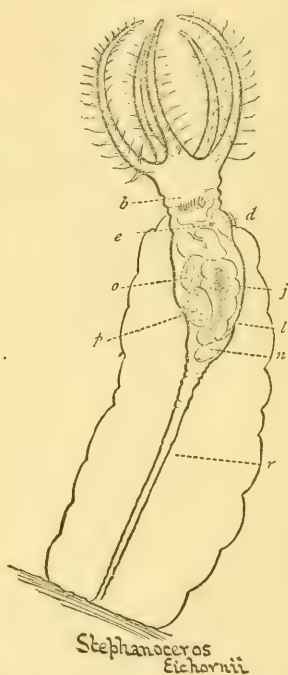


FIG. 9.—*a*. Principal ciliary wreath. *b*. Secondary ditto. *c*. Antenna.

FIG. 10.—*b*. Secondary ciliary wreath. *d*. Cephalic ganglion. *e*. Eye. *j*. Stomach. *l*. Anus. *n*. Contractile vesicle. *o*. Ovary. *p*. Ovum. *r*. Foot.

Worse than this, *Stephanoceros* (fig. 10) and *Floscularia* (fig. 11) are placed in the same family with *Melicerta* (fig. 9) and *Limnias*. Now the former pair differ from the latter most

strikingly, in the shape of the trochal disc, in the disposition of the vibratile cilia, in the position of the mouth, and in the form of the jaws; and it is difficult to understand how Ehrenberg could have persuaded himself to place them together.

Again, *Lacinularia*, *Megalotrocha*, and *Conochilus* all find themselves in different families; though the two former are so alike as to be at times mistaken for each other, and the latter (though its parts are arranged in an unusual manner) is certainly more nearly akin to them than to any other genera. *Conochilus* is indeed a tough morsel for a classifier. In all

FIG. 11.

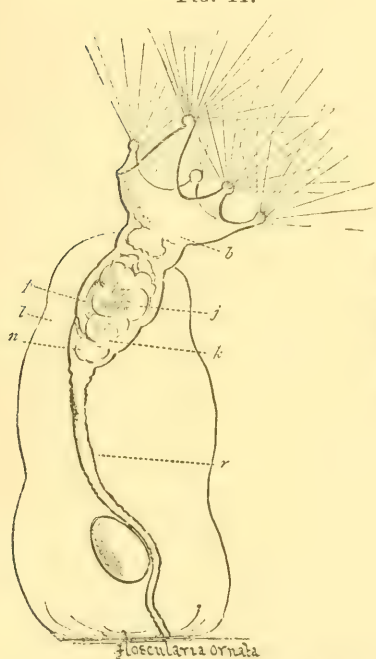


FIG. 12.

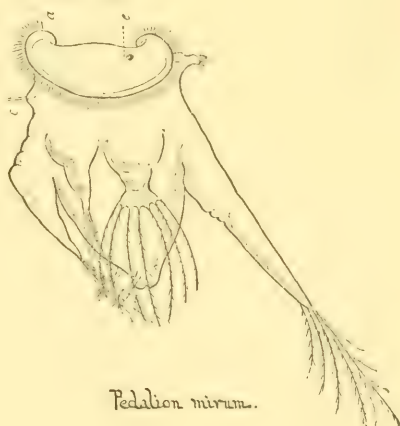


FIG. 11.—*b*. Secondary ciliary wreath. *j*. Stomach. *k*. Intestine. *l*. Anus.  
*n*. Contractile vesicle. *p*. Ovum. *r*. Foot.  
 FIG. 12.—*a*. Principal ciliary wreath. *c*. Antenna. *e*. Eye.

other Melicertans the row of smaller cilia encloses the row of larger ones and also the mouth—the antennæ being outside

of both rows ; but in *Conochilus* all this is reversed ; the row of larger cilia encloses that of the smaller ones, the mouth, and also the antennæ.

Ehrenberg's next great group, the *Sorotrocha*, with its divisions and subdivisions, is more successful ; for the trochal discs have to a considerable degree the characters assigned to them, and the "loricated" families really have loriciæ. The families too are in the main natural ; and two of them, viz. the

FIG. 13

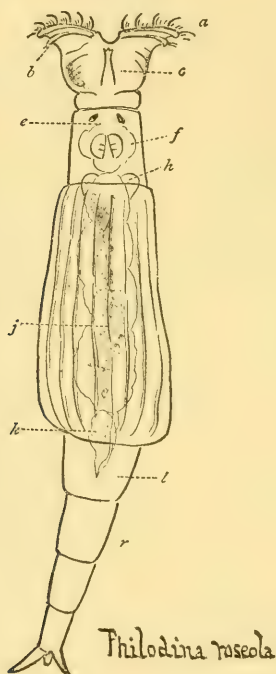


FIG. 13.—*a*. Principal ciliary wreath. *b*. Secondary ditto. *c*. Antenna. *e*. Eyes. *f*. Mastax. *h*. Gastric gland. *j*. Stomach. *k*. Intestine. *l*. Anus. *r*. Foot.

*Philodinæa* and the *Brachionæa*, are so well marked and thoroughly natural, that any system of classification would, I think, leave them almost untouched.



Unfortunately Ehrenberg has thrown these two families into one group—the *Zygotrocha*, and has thus brought together most widely differing forms; for the *Brachionæa* are highly organised Rotifers, while some of the *Philodinæa* are very worm-like, and the structure of the trochal disc, jaw, and foot in the two families is widely unlike.

Of the two remaining families, I think that the *Euchlanidota* should be retained, though its genera require revision; but the other family, the *Hydatinæa*, contains such a number of dissimilar creatures that nothing can save it from subdivision. A glance at such a motley group as *Hydatina senta* (fig. 1), *Notommata aurita* (fig. 4), *Triarthra longiseta* (fig. 8), *Synchæta mordax* (fig. 3), *Pedalion mirum* (fig. 12), and *Asplanchna Brightwellii* (fig. 2), would be enough to make one hesitate to include them in one family; and when it is found that they differ greatly in their internal structure, as well as in their outward form and habits, it becomes tolerably certain that this very large and heterogeneous family cannot be retained as it is.

To sum up then, we may safely say that the majority of the Rotifers in the first great section, the *MONOTROCHA*, do form a distinct and fairly natural group; but that its subdivisions, the *Holotrocha* and the *Schizotrocha*, cannot be maintained, while its families must be altered and the genera re-arranged.

The other great section, the *SOROTROCHA*, must be abandoned; as containing animals that by no means resemble each other in the way that those of the *MONOTROCHA* do: and its subdivisions, the *Polytrocha* and *Zygotrocha*, are equally faulty; uniting dissimilar families such as the *Philodinæa* and *Brachionæa*, while separating similar ones, as the *Brachionæa* and *Euchlanidota*. The families, the *Philodinæa*, *Brachionæa*, and *Euchlanidota*, will in the main hold their ground, but the *Hydatinæa* must be split up and rearranged.

## LEYDIG'S CLASSIFICATION.

Leydig based his classification on the Rotifer's external form, and on the presence or absence of the foot, as well as on the foot's shape and length. As he quite disregarded the whole of the internal structure, as well as that of the trochal disc, it is not to be wondered at that his arrangement is a bad one. The first of these three primary divisions brings together, on account of their shape, such dissimilar creatures as *Melicerta* (fig. 9), *Dinocharis*, *Synchæta* (fig. 3), and *Philodina* (fig. 13)—animals differing alike in habits and internal structure, and only faintly resembling each other in shape. His second primary division, instead of containing any of the great natural groups, simply picks out a few species on account of their sac-like shape, and throws together *Notommata clavulata*, *Polyarthra platyptera*, *Diglena lacustris*, and *Asplanchna Brightwellii*, Rotifers that have hardly one feature in common. His third primary division, containing the *Brachionæa* and *Euchlanidota* is a reasonable one enough; and of his eleven families four are natural, but the rest are so unsuccessful that I propose to pass over his attempt without further comment, while at the same time fully admitting the great value of his observations and researches. It would be doing Leydig the greatest injustice to judge of the rest of his work from his classification of the animals that he so successfully studied.

## DUJARDIN'S CLASSIFICATION.

Of Dujardin I must speak in very different terms. His book is mainly critical; and, so far as I can find, contains little on the Rotifers that was new, except his observations on *Albertia* and *Lindia*.

His criticisms are shrewd, and often just; he points out that Ehrenberg's respiratory tube is probably an antenna, and suggests that the convoluted tubes with their flickering tags and contractile vesicle are a respiratory system; an erroneous

suggestion, I believe, but one that has found wide acceptance. On the other hand, he could not see *Floscularia*'s (fig. 11) tube, could not make out the striated muscles in any Rotifer, could see no difference between the muscles and the nerves, and doubted the existence, as specialised structure, of either the one or the other. He denied, also, that there was good reason for believing that any of the red spots were eyes.

But although he has small claim to be considered either an original or an accurate observer of the Rotifers, his classification has one happy hit. He formed his primary groups according to their various modes of locomotion. This produces three orders—Rotifers that are fixed; those that swim only; and those that both swim and creep like a leech. The first includes the *Floscules* and *Melicertans*; the second, the *Brachionæ*, *Euchlanidota* and *Notommata*; and the third, the *Philodinæa*. The arrangement is excellent, and requires only to be supplemented by the addition of a fourth group to contain Rotifers (like *Pedalion mirum* (fig. 12)), which not only swim, but also skip by means of real limbs.

In the details of his system Dujardin often fails, and obviously from lack of personal acquaintance with the forms he is classifying. For instance, he places *Æcistes* and *Conochilus* in the same genus, declaring that the only important difference between them is their tube.

I have already pointed out above how widely the structure of *Conochilus* differs from that of *Melicerta* (fig. 9), and how closely that of *Æcistes* agrees with it. Dujardin could not have made a more unfortunate selection of two Rotifers to form a genus with. He follows Ehrenberg in placing *Tubicolaria* (a form of the ordinary *Melicertan* type) in a genus by itself; and he places in the same genus *Hydatina* (fig. 1) and *Synchæta* (fig. 3), genera whose trochal discs, jaws, alimentary canals, and vascular systems are widely unlike. On the whole however his system has great merit; and would have had much more, had his knowledge of details been at all commensurate with his critical faculty.

## DR. BARTSCH'S CLASSIFICATION.

Dr. Bartsch, in his first publication of his system in 1870, divided all the Rotifers into the Enterodela (with stomach, intestine, and anus) and the Gasterodela (without intestine or anus); and in this latter was one family formed for one genus *Ascomorpha* (Gosse's *Sacculus*); all the rest of the Rotifers were in the first division.

In his second publication, '*Rotatoria Hungariæ*,' he abandoned this primary division, and simply arranged the Rotifers in six families, as follows :

1. Floscularinæ.
2. Philodinæa.
3. Hydatinæa.
4. Longisetæ.
5. Scaridina.
6. Loricata.

Of these 1, 2, and 6 are natural, though 1 is made to contain *Floscularia* (fig. 11) and *Stephanoceros* (fig. 10) along with the *Melicerians*; and the first two differ too much from the last to be so placed.

Family 3 ranks together *Hydatina* (fig. 1), *Synchaeta* (fig. 3), *Asplanchna* (fig. 2), and *Lindia*; four forms that ought to be in separate families: while family 4 connects the dissimilar genera *Triarthra* (fig. 8), *Mastigocerca*, *Polyarthra*, and *Furcularia*.

This system is much on a par with Leydig's, but the publication in which it occurs contains plates which though coarse are well worth attentive study. I can say nothing of the text, which is unfortunately in a language that I cannot read.

Having thus discussed the four rival systems, I propose next to offer my own attempt at a reclassification of the Rotifers. Of course I can lay little claim to originality, and cannot pretend to do much more than select and combine the best thoughts of my predecessors. I have availed myself of Dujardin's orders, and of Leydig's use of the foot, and I have left



Ehrenberg's genera in the main unaltered; I have also made ample use of that mine of information, the essay on the Rotifera,

FIG. 14.

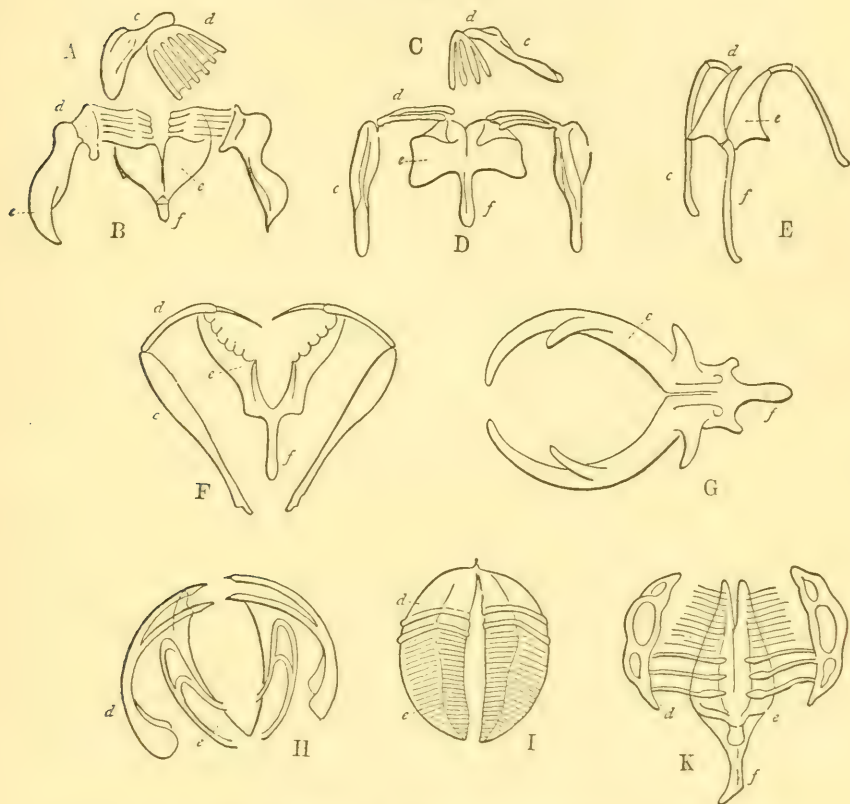


FIG. 14.—*A.* Single malleus of *Brachionus urceolaris*. *B.* Trophi of *Brachionus urceolaris*. *C.* Single malleus of *Euchlanis deflexa*. *D.* Trophi of *Euchlanis deflexa*. *E.* Trophi of *Notommata petromyzon*. *F.* Trophi of *Diglena forcipata*. *G.* Trophi of *Asplanchna priodonta*. *H.* Trophi of *Stephanoceros Eichornii*. *I.* Trophi of *Philodina roseola*. *K.* Trophi of *Limnias Ceratophylli*. *c.* Manubrium. *d.* Uncus. *c* and *d.* Malleus. *e.* Ramus. *f.* Fulcrum. *e* and *f.* Incus. All after Gosse, except *G* and *H*.

in Pritchard's 'Infusoria' (1861), as also of Gosse's admirable

though unfinished sketch in vol i and ii of the 'Popular Scientific Review' (1862 and 1863); and finally I have adhered to the utmost to the old nomenclature, and endeavoured to meddle as little as possible with the great Prussian naturalist's original plan.

The class Rotifera falls then, I think, into four natural orders according to their modes of locomotion. There are some that swim only; others that both swim and creep like a leech; those that both swim and skip; and lastly, those that when adult are fixed: and these orders differ in the main from each other in the form and use of the foot.

In each order too there are typical genera, round which the rest may be grouped, differing from each other in the shape of the trochal disc, and the position of its ciliary wreaths, as also in the structure of the manducatory organs; and sometimes in other important points as well.

But before I describe the families that can be formed round these typical genera, I must digress a little to explain certain technical names which I shall find it necessary to use.

Gosse's exhaustive treatise on 'The Manducatory Organs in the Class Rotifera,' enables us to see that these organs present seven or eight types of structure, distinguished from each other by the prominence of some particular part.

To make this clear it may be as well to state that in the typical mastax of a *Brachionus* there are two hammer-like bodies (mallei) (fig. 14, *B*; *c*, *d*), which work on a kind of split anvil (incus) (fig. 14, *B*, *ef*); and that each malleus consists of an upper part or head (uncus) (fig. 14, *B*, *d*), and a lower or handle (manubrium) (fig. 14, *B*, *c*); while the incus also consists of two, the upper divided into two symmetrical halves (rami) (fig. 14, *B*, *e*), which are supported on the lower piece (fulcrum) (fig. 14, *B*, *f*); these hard portions of the mastax are termed the trophi.

Now, in *Brachionus* (fig. 7) all the trophi are well developed; but the other typical manducatory organs may be arranged in a series in which the mallei are successively degraded, while continually greater prominence is given to the incus; at least

in all but three, and in two of these the rami and unci are the prominent parts, while the third is distinguished by the close connection of the mallei and rami.

The typical trophi then may be named as follows :

#### 1. Malleate.

Mallei stout ; manubria and unci of nearly equal length ; unci 5- to 7-toothed ; fulcrum short ; as in *Brachionus urceolaris*, Fig. 14, A, B.

#### 2. Sub-Malleate.

Mallei slender ; manubria about twice as long as unci ; unci 3- to 5-toothed ; as in *Euchlanis deflexa*, Fig. 14, C, D.

#### 3. Virgate.

Mallei rod-like ; manubria and fulcrum very long ; unci 1- or 2-toothed ; as in *Notommata petromyzon*, Fig. 14, E.

#### 4. Forcipate.

Mallei rod-like ; unci pointed or evanescent ; rami much developed, and used as a forceps ; as in *Diglena forcipata*, Fig. 14, F.

#### 5. Incudate.

Rami highly developed into a curved forceps ; mallei evanescent ; fulcrum stout ; as in *Asplanchna priodonta*, Fig. 14, G.

#### 6. Uncinate.

Unci 2-toothed ; manubria evanescent ; incus slender ; as in *Stephanoceros Eichornii*, Fig. 14, H.

#### 7. Ramate.

Rami sub-quadrantic, each crossed by 2 or 3 teeth ; manubria evanescent ; fulcrum rudimentary ; as in *Philodina roseola*, Fig. 14, I.

## 8. Malleo-ramate.

Mallei fastened by unci to rami; manubria 3 loops soldered to the unci; unci 3-toothed; rami large, with many striae parallel to the teeth; fulcrum slender; as in *Limnias Cera-tophylli*, Fig. 14, κ.

Now, if we leave out *Diglena forcipata*, the other examples

FIG. 15.



FIG. 15.—1. Male of *Floscularia campanulata*. 2. Male of *Lacinularia socialis*. 3. Male of *Notommata Brachionus*. 4. Male of *Synchaeta tremula*. 5. Male of *Asplanchna Ebbesbornii*. 6. Male of *Brachionus urceolarias*, copied from a drawing by Mr. P. H. Gosse, F.R.S. 7. Male of *Salpina mucronata*, from a drawing by Mr. E. C. Bousfield. 8. Male of *Pedalion mirum*. s. Sperm-sac. p. Penis. c. Contractile vesicle.

of the typical trophi give us seven rotifers very distinct from



each other; and show that the form of the trophi is a good characteristic for separating the families. But a difference in the shape and disposition of the trochal disc and its ciliary wreaths generally accompanies a difference in the manducatory organs; and the two together will, I think, serve as good guides to a re-classification of the Rotifers into families. This I have attempted in the annexed scheme, but of course there are genera which do not fall readily into this arrangement; such aberrant forms as *Trochosphaera*, *Acyclus*, and *Dictyophora*, it would be difficult to place in any classification.

The parasitic Rotifers (as might have been expected) contain some very strange creatures, such as *Drilophaga* and *Seison*; and would I think be better put in a class by themselves. Such difficulties however must attend every attempt to marshal Nature's endless varieties into well-marked battalions. Nature knows no hard lines of separation, and the best of classifications can be only that which contains the fewest faults.

Perfectly satisfactory classification is the product of imperfect knowledge; when the commoner and better separated forms are alone known to us, and when the rarer intermediate forms (which are the despair of the classifier and the delight of the naturalist) are as yet undiscovered.<sup>1</sup>

## CLASS—ROTIFERA.

### Order I.—RHIZOTA.

Fixed forms; foot attached, transversely wrinkled, non-retractile, truncate.

#### Fam. 1. Flosculariadae (figs. 10, 11).

Mouth central; ciliary wreath a single half-circle above the mouth; trophi uncinatæ.

<sup>1</sup> Some years ago it was thought that the Rotifers might possibly be divided into two groups; the one monœcious, the other diœcious. But later researches have rendered this improbable. For, of the twelve families into

## Fam. 2. Melicertadæ (fig. 9).

Mouth lateral; wreath two marginal curves nearly surrounding the head with mouth between; trophi malleoramate.

## Order II.—BDELLOIDA.

That swim and creep like a leech; foot retractile, jointed, telescopic, termination furcate.

## Fam. 3. Philodinadæ (fig. 13).

Trochal disc two transverse circular lobes; wreath two marginal curves on each lobe with mouth between; or trochal disc of one lobe ventrally furred with cilia; trophi ramate.

## Order III. PLÖIMA.

That only swim.

\* Il-loricated.

## Fam. 4. Hydatinadæ (fig. 1).

Trochal disc transverse with ciliated prominences; wreath double; trophi malleate; brain small, not sack-like; foot furcate.

## Fam. 5. Synchætadæ (fig. 3).

Trochal disc rounded; wreath of interrupted curves, surrounding the head; trophi virgate; foot absent, or minute.

## Fam. 6. Notommatadæ (fig. 4).

Trochal disc oblique; wreath of interrupted curves and clusters; trophi virgate or forcipate; brain large, sack-like; foot furcate.

which I have divided the Rotifera, no less than eight contain species of which the males have been seen (see Fig. 15); and in the remaining four the sexual organs exactly resemble those of the females in the other eight families. That males will be ultimately discovered in the four families where they are at present unknown, I have little doubt.

Fig. 15 contains the male of one species only in each of the above eight families; but the males of many more than these have been observed and figured.

## Fam. 7. Triarthradæ (fig. 8).

Trochal disc transverse; wreath single, marginal; trophi malleo-ramate; foot absent.

## Fam. 8. Asplanchnadæ (fig. 2).

Trochal disc rounded; wreath single, marginal; trophi incudate; intestine, anus, and foot absent.

\* \* Loricated.

## Fam. 9. Brachionidæ (fig. 7).

Trochal disc transverse with ciliated prominences; wreath single, marginal; trophi malleate; lorica entire, simple; foot transversely wrinkled, wholly retractile, 2-toed or absent.

## Fam. 10. Pterodinadæ (fig. 6).

Trochal disc two transverse circular lobes; wreath on each double, marginal; trophi malleo-ramate; foot transversely wrinkled, wholly retractile, ending in a ciliated cup.

## Fam. 11. Euchlanidæ (fig. 5).

Trochal disc rounded; wreath in interrupted curves, and clusters; trophi sub-malleate or virgate; lorica in two parts, meeting in a furrow, or entire with additional pieces; foot jointed, feebly retractile, not telescopic or transversely wrinkled—furcate or stylate.

## Order IV.—SCIRTOPODA.

That swim with their ciliary wreath, and skip by means of hollow limbs with internal locomotor muscles.

## Fam 12. Pedalionidæ (fig. 2).

Trochal disc transverse; wreath two marginal curves with mouth between; trophi malleo-ramate; foot replaced by two posterior ciliated processes.

GENERA.<sup>1</sup>

1. Flosculariadæ . . Floscularia (fig. 11), Stephanoceros (fig. 10).
2. Melicertadæ . . Melicerta (fig. 9), Limnias, Œcistes, Cephalosiphon,  
Lacinularia, Megalotrocha, Conochilus.
3. Philodinadæ . . Philodina (fig. 13), Rotifer, Callidina.
4. Hydatinadæ . . Hydatina (fig. 1), Rhinops.
5. Synchætadæ . . Synchæta (fig. 3), Polyarthra.
6. Notommatadæ . . Notommata (fig. 4), Diglena, Furcularia, Scaridium,  
Pleurotrocha, Distemma.
7. Triarthradæ . . Triarthra (fig. 8).
8. Asplanchnadæ . . Asplanchna (fig. 2).
9. Brachionidæ . . Brachionus (fig. 7), Noteus, Anuræa, Sacculus.
10. Pterodinadæ . . Pterodina (fig. 6), Pompholyx.
11. Euchlanidæ . . Euchlanis (fig. 5), Salpina, Diplax, Monostyla, Co-  
lurus, Monura, Metopidia, Stephanops, Monocerca,  
Mastigocerca, Dinocharis.
12. Pedalionidæ . . Pedalion (fig. 12).

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<sup>1</sup> The principal ones ; several of Ehrenberg's are omitted for various reasons that cannot here be detailed ; and the genus Notommata, though the name is retained, is here supposed to have lost a large number of Ehrenberg's species.

The thirteen figures, given as illustrations of the various families, are not drawn on the same scale ; and no attempt has been made to show in them all the details of internal structure.

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## Further Observations on Flagellated Organisms in the Blood of Animals.

By

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IN a memoir on "The Microscopic Organisms found in the Blood of Man and Animals," published in the 'Fourteenth Annual Report of the Sanitary Commissioner with the Government of India,' and which was, in great part, reproduced in the first three numbers of the 'Quarterly Journal of Microscopical Science' for 1879, a chapter was devoted to a description of certain flagellated organisms which I had found in the blood of rats. This chapter will be found in the January number at p. 109, so that it will not be necessary on the present occasion to do more than very briefly recapitulate what was given therein.

Having been directed to make certain inquiries regarding the nature of the, sometimes designated, "spirillum-fever" which prevailed in Bombay during the earlier part of 1877, I had occasion to examine the blood of a considerable number of animals, and in July of that year detected spirillum-like organisms in the blood of healthy rats. In some instances these were so numerous that the blood when examined under a high power seemed to quiver with life. On careful focussing it was ascertained that each organism consisted of a body-portion and of an extension of it in the form of a gradually tapering, long flagellum, the former average  $25\ \mu$  in length by  $1\ \mu$  in width, whilst the flagellum brought up the total length of the organism to about  $50\ \mu$  or longer, for it was by no means certain that the whole length of the free end of the flagellum

was visible. They were found not to be very sensitive to reagents, as they continued, for example, to manifest lively movements in a weak solution of bichloride of mercury for eight hours, and an exposure of several minutes to chloroform vapour did not seem to affect them. A weak solution of ammonia did not affect them for some time, but a stronger solution of potash affected them at once. When a drop of blood containing them was placed on a slide arranged for the application of electricity, it was found that an interrupted current of such a strength as could not be comfortably borne by an individual was tolerated by these beings for several consecutive hours.

They were found in two species of rats—*Mus decumanus* and *Mus rufescens*—and in 29 per cent. of the animals examined. At that time I had not specially searched for these organisms anywhere except in Calcutta, nor had I found them in the blood of any animal except in that of the rat. I have since found them in rats at Simla, in the Himalayas, at an elevation of 7500 feet above sea-level, though as regards the blood of mice and of musk rats I have searched for them in vain both in Simla and Calcutta.

That they are, however, to be found in the blood of other animals has been demonstrated by Dr. Griffith Evans, the present chief of the veterinary department in Madras, who, in 1880, whilst examining the blood of horses suffering from a wasting form of disease termed “surra” in the Punjab, found that it frequently swarmed with organisms of this character. Dr. Evans further made the very interesting observation that in the blood of a couple of camels, suffering apparently from a disease allied to surra in the horse, flagellated organisms were present in one, and nematoid embryos, closely resembling those which I described some years ago as being found in the blood of man, the *Filaria sanguinis hominis*,<sup>1</sup> in the other. I have elsewhere<sup>2</sup> drawn attention to this parasite of the camel,

<sup>1</sup> ‘On a Hæmatozoon in Human Blood; Its relation to Chyluria and other Diseases.’ Calcutta: Office of Superintendent of Government Printing, 1872

<sup>2</sup> ‘Proceedings of the Asiatic Society of Bengal,’ March, 1882.

parents and embryos, and suggested that it might be called *Filaria Evansi*. I hope, however, to describe it at greater length in the next number of this Journal.

With the view of ascertaining whether these flagellated organisms could be transferred to other animals, Dr. Evans had injected some blood from a horse, in which these organisms abounded, into the subcutaneous tissue of a dog and of a bitch, and on examining their blood four or five days afterwards precisely similar organisms were found in the blood of the bitch, but not in that of the dog. This bitch had a suckling puppy about a couple of months old, and its blood also contained these organisms, although it had not been intentionally inoculated; though as regards the possibility of the puppy having likewise been inoculated from the horse it is to be mentioned that a little of the blood was given to the bitch to eat, and it is quite possible that the puppy likewise consumed some of this. Unfortunately, the blood of these animals had not been examined as a preliminary procedure, so that it cannot be definitely declared that the organisms had been derived from the blood of the horse. It is just possible that they may have existed in their blood previously, and, in this connection, it is to be borne in mind that as regards rats attention was drawn in my previous article to the circumstance that the blood of those caught in a particular room would be affected, "whereas the blood of rats in another part of the building would not contain them. The servants had ultimately come to recognise this, as, whenever they learnt that a particular rat's blood contained the desired organisms, they diligently endeavoured to secure the rest of the family," so that the possibility is not absolutely excluded that the finding of these parasites in the blood of the puppy and of its mother may have been a coincidence and not the direct result of the experiment; nor is it known to what extent the blood of horses and camels or other animals in this part of India may harbour these organisms or may have harboured them at that time.

These flagellated blood-parasites are not, however, limited to India, for in 1881 Wittich described similar organisms in the

blood of hamsters in Germany.<sup>1</sup> Wittich's experience coincided with my own as regards their being found in the blood of apparently perfectly healthy animals, though Dr. Robert Koch,<sup>2</sup> instigated by the result of Wittich's observations, found that the hamsters which he procured died, one within two days of being in captivity, and four others subsequently. It does not appear that the blood of these hamsters was examined during life, but after death it was found, in each case, to contain the organisms in question. No reference is made to the examination of other hamsters, so that it is not quite clear whether the animals died as a result of captivity or in consequence of the parasitism. As regards rats thus affected I have had them kept in a cage for weeks, and to all appearances in a state of perfect health. Both Wittich and Koch suggest that the parasites found by them in the blood of hamsters are in all probability identical with those found by me in rats in India; and Koch gives two micro-photographs of them which correspond very closely with the micro-photographs which were published by me in the above-mentioned Indian 'Sanitary Report.'

What these organisms are and whence their origin is by no means clear, and as the suggestions which have been offered by various authorities regarding these points are so greatly at variance it seems highly desirable that every detail which can be collected concerning them should be placed on record. This is all the more to be desired, seeing that the question has arisen of their possible influence as a cause of disease.

I had every opportunity of satisfying myself that the parasite found by Dr. Evans in the dog is identical with that in the rat, as Dr. Evans brought the puppy to Simla in October, 1880, and very kindly made it over to me for observation. The accompanying sketch represents some of the forms assumed by these organisms as observed under a Prazmowski's 1.5 mm. immersion objective. This, together with the following remarks made at the time, are copied from my note-book :

A drop of blood having been obtained from the puppy's ear

<sup>1</sup> 'Centralblatt für die medicin. Wissensch.,' vol. xix, No. 4.

<sup>2</sup> 'Mittheilungen aus dem Kaiserlichen Gesundheitsamte,' vol. i, p. 9, 1881.



about 9 a.m. on the 26th October, it was found to contain a considerable number of these organisms in a state of great activity.

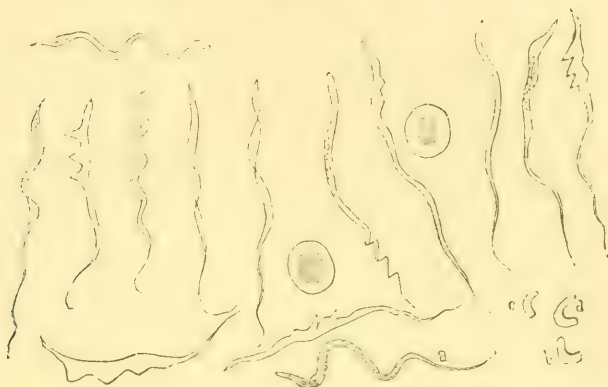


FIG. 1.—Various appearances presented by flagellated organisms in blood from a puppy's ear, seven to eight hours after it had been under the cover-glass: at *a* after eleven hours. *b*, *c*, *d*, changes undergone by a proto-plasmic body in the field during the course of a few minutes. Two blood-corpuscles introduced to indicate the relative size.  $\times 1000$  diameters.

Their movements were so rapid that it was impossible to obtain a clear conception of their exact form. The slide was set aside and again examined at 4 p.m., when it was found that their movements were much more languid and less suggestive of spirilla than they were in the morning. It was at this time that the figures above reproduced were sketched. As the rapidity of the movements diminished there appeared to be a greater tendency to throw out flagellæ, and wave-like extensions of their substance seemed to originate at the thicker end and to pass along rapidly towards the flagellum. The plasma-substance appeared to be contractile along the whole length of the parasite, even to the very tip of the flagellum, and, frequently, an impression was conveyed, suggestive of the organism being flat or ribbon-like; consequently, when seen in profile they presented a much more attenuated aspect than under other conditions. Moreover, it is difficult to decide how much of the wave-like appearance above referred to is due to rapid

lateral prolongations of the protoplasm, and how much to the aspect which would be presented when this ribbon-like form undergoes rapid spiral contractions. In the above figures an attempt has been made to reproduce the most striking of these different appearances, and a couple of red blood-corpuscles have been introduced to indicate the relative size under the same magnifying power.

At 5 p.m. the organisms manifested signs of losing vitality, became more ribbon-like and pointed—almost, if not quite, ‘lashed,’ at the thicker end also; moreover, a clear space is observed at a distance of from 2 to 3  $\mu$  from the point highly suggestive of a vacuole. This is indicated in two or three of the figures. They averaged about 30  $\mu$  in length and from 1 to 2  $\mu$  in width at the thickest part.

At 8 p.m. only two specimens could be detected in the slide, one quite motionless, the other nearly so. One of these is carefully sketched at *a* in the engraving; a vacuole-like spot is observable at one part, and the parasite is granular almost along its entire length. Near this specimen a curved protoplasmic object was observed to alter its form very slowly, as shown at *a, b, c*. Its further changes could not be followed, as it was lost whilst its form was being outlined, but I have on two or three occasions observed objects of this character associated with these parasites, and sometimes think that they must represent either an earlier or a later stage of them than is ordinarily seen.

Further specimens of blood were obtained from the puppy on the 29th and 30th of October, but no organisms could be detected; on the 3rd November, however, it is noted that the organisms were very numerous in the blood, and that “the dog looks remarkably well.”

Shortly afterwards the puppy was taken to Calcutta, and when examined on the 25th November no organisms could be detected in its blood. On the 3rd December, however, they were readily found. A specimen which was observed on this occasion may serve to illustrate a phenomenon which I have frequently observed in connection with like organisms in the

rat. A slide of blood which had been kept in a moist chamber for twenty hours having been placed under the microscope, the eye was attracted by the way in which one of the parasites appeared to play with a red blood-corpuscle. It was watched for fully an hour, until, in fact, the field was disturbed by the evaporation along the edge of the cover-glass. Its movements were sluggish and just sufficient to slightly shift the corpuscle. It had not attached itself to the corpuscle by either of its ends, but at a spot about  $8\ \mu$  from the point of the thicker end as shown in Fig. 2, *a* to *d*. Sometimes there appeared to be a slight interval between the corpuscle and the parasite (Fig. 2, *a*), and occasionally even a greater interval than is indicated in the woodcut, but both parasite and corpuscle, nevertheless, continued to move in unison, as though some filamentous connection existed between them, which, however, was too delicate to be distinguished by the highest power which I possessed. At other times the organism appeared to be closely applied to the corpuscle, as though the latter were being embraced by two short lateral pseudopods, and the outline of corpuscle appeared as if squeezed (Fig. 2, *b*, *c*). At Fig. 2, *d*, the corpuscle is shown with the parasite immediately below it. No

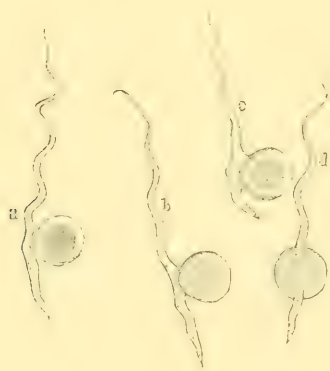


FIG. 2.—*a—d* appearances presented by one of the flagellated organisms which had applied itself to a red blood-corpuscle.  $\times 1000$  diameters.

distinct flagellum could be detected extending from the thicker

portion of the parasite, though it was frequently observed to present a ribbon-like appearance.

Shortly after this the puppy got the "distemper," and was struck by one of the native servants, so that it lost the sight of the right eye. On the 15th January, 1881, several specimens of blood from its ear were examined, but not a single parasite could be detected. Further examinations were made on the 12th and 28th February, and again on the 24th March, but not a single specimen was found. The dates have been carefully recorded, as they may be of use to future observers; and the notes of observations in this instance have been made to subserve the double object of illustrating the more salient points in the microscopy of the parasite, and to give the exact history of this form of parasitism in the dog during a period of from four to five months.

There are, however, a few other points in connection with the microscopy of these organisms which it seems desirable to refer to as they may be of assistance to systematic writers in deciding their precise position in the animal (or, as some authorities may perhaps consider, vegetable) kingdom.

These supplementary details will be based on further observations which have been made from time to time, as opportunities offered, during the last three or four years, on the blood of rats, but more particularly on a series which were conducted for purposes of comparison whilst the organisms in the dog were being watched.

On January 30th, 1881, the following entry is made in my note-book: Examined the blood of five rats, and found flagellated organisms in two of them. One of the latter was a pregnant female, but this one, however, did not contain many specimens of the parasite, and none were found in the blood of its young. The blood of the other rat swarmed with the organisms.

As it had been found that the parasites were remarkably well preserved in a 0.75 per cent. solution of salt and water, half a Pravaz-syringeful of a mixture of the blood of this rat, and of the salt solution—one part to three—was injected into the



sub-cutaneous tissue of the thigh of a healthy rat, free from blood organisms, and which had been under observation for a fortnight.

The animal did not appear to be materially affected by this procedure, and on February 12th it is recorded: The rat continues to enjoy excellent health; eats and drinks freely. Not a trace of any organisms found in its blood, although the flagellated organisms which had been introduced into its tissues were found to be alive two days after the operation in what remained of the mixture which had been injected.

It would thus appear that these organisms are at all events not very readily transmissible by means of sub-cutaneous injection from one rat to another.

Nor have I succeeded in preserving them beyond two or three days outside the body. Attempts have been made to "cultivate" them in plain water, in sugar and water, glycerine and water, and in salt and water, as, also, in the blood itself, both with and without the aid of an incubator. But I could not satisfy myself that they multiplied; on the contrary, they seemed to degenerate after removal from the animal hour by hour. A weak solution of salt, as already observed, appeared to be a more favorable medium for retaining their vitality than

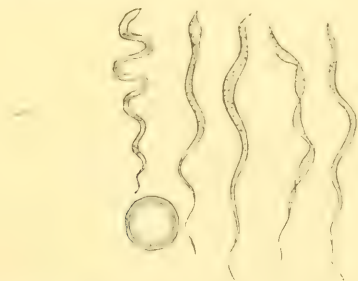


FIG. 3.—Flagellated organisms from the blood of a rat preserved in a 0.75 per cent. solution of common salt.  $\times 1000$  diameters.

any other which I have tried, and is a very convenient medium for studying the various stages of the disintegrative process.

The different appearances which they presented as watched in such a solution are sketched at Fig. 3; from which it will be observed that the organisms present a striking resemblance to the more generally recognised forms of spermatozoa. On the third day the specimens figured were no longer recognisable in the fluid in which they were kept.

Whilst watching these particular specimens I was further able to satisfy myself that these, like the generality of flagellated organisms, moved with the lash in front—that is to say, in the direction indicated by the arrow which is placed alongside of the middle specimen in Fig. 4. Since this period I have frequently observed the same thing in other specimens, though it is scarcely possible to be sure of the direction of the movement until after the parasite has become sluggish. Moreover, they may also be observed to move with the thicker end forwards, but only for short distances.

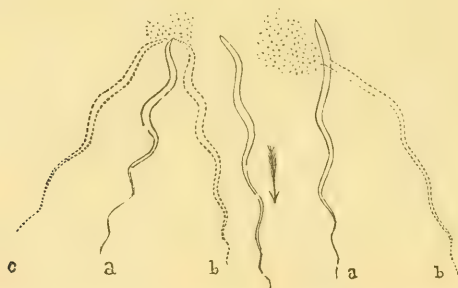


FIG. 4.—Different methods of attachment to foreign bodies observed in two specimens of the organisms from the blood of a rat. The arrow along the middle figure indicates the direction of progressive movement.  $\times 1000$  diameters.

As already remarked, when describing the specimens from the blood of the dog, they seem to attach themselves to surrounding objects by means of some portion of the thicker end. The specimen sketched in the left half of Fig. 4 was observed to remain attached to a granular mass by the extreme point of its thicker end for a considerable time, whilst the remainder of the

parasite was seen to swing freely from *a* to *b* and from *a* to *c*, the free end of the lash presenting a screw-like appearance. Another specimen was watched for half an hour whilst it remained attached to a granular mass in the preparation, but in this instance, as represented at the right half of Fig. 4, the parasite had fixed itself at a point about  $3\ \mu$  from the end, and to swing itself, as from *a* to *b*, from this fixed position. It will be noted that the part of the body by which the parasite attaches itself here corresponds with that represented as having been attached to the red blood-corpuscle in a previous figure. In this instance, also, the lash was observed to manifest incessant screw-like movements, the movement apparently commencing at the tip of the flagellum and proceeding rapidly upwards until the point of attachment to the granular mass was reached, and here it stopped abruptly.

Many attempts were made to demonstrate the presence of another flagellum at the opposite end, but without any satisfactory result. In preparations made by drying a film of the affected blood on a cover-glass both ends of the parasite are often seen to be very pointed, but in all cases a distinct flagellum could only be made out at one end. When a solution of gentian-violet is added to such a slide the parasites are rapidly stained and present a granular appearance throughout, granules being frequently distinguishable as far as the extreme tip of the flagellum, as may be observed in Fig. 5. Occasionally the



FIG. 5.—Action of gentian-violet on specimens of the organisms from the blood of a rat.  $\times 1000$  diameters.

flagellum appears to be retracted, as shown in the sketch in the middle of the figure, and I have sometimes thought that such a

retraction of the flagellum could be observed whilst the organism was in a condition of extreme activity. The specimens in this figure were carefully outlined to scale by means of the camera lucida.

I am wholly unable to suggest any explanation for the presence of these flagellated parasites in the blood of animals. It will be recollected that they have now been observed in the blood of the horse, camel, and hamster, in addition to that of the rat; and, further, that they have been found in the blood of two dogs, but whether as the result of intentional inoculation or otherwise must for the present be left undecided. As regards the season in which they may be detected, I find that there are entries in my note-book of their having been seen, at one time or another, in the blood of rats in nearly each month of the year.

For some time I was inclined to think that they might be the spermatozoa of some parasite hidden in the tissues of the animal, a view which strongly forced itself upon me some years ago, in 1878, by having accidentally observed a large number of spermatozoids escaping from the reproductive pore of a fragment of *tænia* which I had found while dissecting a rat. The "head" of the *tænia* was not found, so that the entozoon could not be identified with certainty, but it probably was a portion of *Tænia microstoma* or some closely allied species. My notes run as follows:—The segments having been placed on a slide spermatozoids are seen to escape from the genital pore of nearly every one of them. For a few moments after their escape they presented, with amazing exactness, the characters of the spirillar organisms found in the blood of rats, but which were not present in the blood of this particular specimen. It seemed, however, that the water in which the *tænia* segments were mounted and into which they escaped was not suitable to their preservation. They rapidly underwent changes of form, and almost before half a dozen of them could be sketched disintegrative changes set in, and the previously active flagellated organisms were transformed into quiescent, filamentous shreds. It has not been considered



necessary to reproduce the sketches of them which were made, seeing that both as to size and form they are so very like several of the figures in the woodcuts already given. The organisms found in the blood of rats, however, are by no means so sensitive to the action of water as this.

Leuckart, in his recently published review of the additions which have been made during 1876 to 1879 to the literature of low forms of animal life,<sup>1</sup> suggests that it is doubtful whether these rat organisms should not be relegated to the class of organisms described by Dr. Gaule as being present in the blood and spleen of frogs and termed by him "Cytozoa" rather than to the flagellata. Readers of this Journal will recollect that Dr. Gaule was under the impression that these "Cytozoa" (also described by him as "Würmchen") were the result of certain changes which took place in the blood-corpuscles and other cellular elements of frogs. Professor Ray Lankester, however, in the number of this Journal for January, 1882, has shown that such an inference is wholly erroneous, and has, I think, very satisfactorily demonstrated that Gaule's Cytozoa are "independent parasitic organisms"—that they represent, in fact, the young stage of a Sporozoon. It is not quite clear to which view of the nature of these "Cytozoa," to Gaule's or to Lankester's, Leuckart refers in the paragraph above cited.

In his recently completed 'Manual of the Infusoria,' Mr. Saville Kent, on the other hand, has placed the blood-organism of the rat amongst the Flagellata, and has named them *Herpetomonas Lewisi*; at the same time he points out that it is possible that further research "may possibly demonstrate their identity with the discharged spermatid elements of the minute nematodes, micro-filariæ, or other metazoic endo-parasitic forms known to flourish amid the same surroundings."

<sup>1</sup> 'Bericht über die wissenschaftlichen Leistungen in der Naturgeschichte der niederen Thiere,' ii Hälfte, 1883, p. 775.

## Physiology of Protoplasmic Movement.<sup>1</sup>

By

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in Utrecht.

### I. INTRODUCTION.

LIVING protoplasm possesses in very many instances the inherent property of moving with a rapidity which is perceptible with the aid of the microscope.

The movements, which show themselves by a change of form and internal arrangement of the protoplasmic masses while the volume remains apparently unchanged, may be also artificially produced or influenced by what are called stimuli.

The existence of these movements is an intrinsic part of the general conditions of life.

In this they agree with muscular and ciliary movements, with which, indeed, they are closely connected by numerous transitional forms. They must be classed with both the latter varieties of organic movement as phenomena of contractility.

The special character of protoplasmic movements lies in this, that the particles of the contractile mass move, as a rule, not in relation to any fixed position of equilibrium, but can change their arrangement and position (and this apparently voluntarily) as do the moving particles of a fluid substance. Further, the impulse to such movements does not normally come from without, but originates in the moving particles

<sup>1</sup> Translated from Hermann's 'Handwörterb. der Physiologie,' by A. G. Bourne, B.Sc.

themselves. Protoplasm thus possesses, not only contractility and irritability, but also automatism.

Protoplasm, in accordance with its low stage of organisation, unites in itself the three properties which are usually divided between histologically differing elements—in the case of ciliary organs between the protoplasm and the cilia, in the case of muscular organs between the ganglia, the nerves, and the muscles themselves.

With this agrees its remarkably wide distribution in animal and vegetable organisms, its prevalence among the lowest forms of life in both kingdoms, as well as among embryonic or, above all, young cells, and in the same way the absence of a more complicated anatomical structure.

Sharply defined limits between protoplasmic movements and those of other organic structures cannot be drawn. As exhibiting movements transitional between protoplasmic and muscular movement may be instanced the body-substance of numerous Infusoria,<sup>1</sup> the tentacles of Acinetæ, the superficial sarcode of Sponges,<sup>2</sup> embryonic muscle-cells of higher forms, endothelial cells of many, more especially young, blood-capillaries,<sup>3</sup> &c. Pigment cells, which are contractile under the influence of a nerve impulse, found in the skin of Crustacea, Fishes, Amphibia, and Reptiles, may be also mentioned in this connection.<sup>4</sup> Transitions from protoplasmic to ciliary movement, or more especially in the other direction, have been observed by De Bary and others in the spores of Myxomy-

<sup>1</sup> Th. W. Engelmann, "Contractilität u. Doppelbrechung," 'Arch. f. d. ges. Physiol.,' xi, p. 448, 1875.

<sup>2</sup> Lieberkühn, 'Ueb. Bewegungserschein. d. Zellen,' p. 346, Marburg, 1870.

<sup>3</sup> Cf. among others: S. Stricker, "Untersuchungen über die Contractilität der Capillaren," 'Wiener Sitzungsber. d. Math.-naturw.,' Cl. lxxiv, p. 313, 1877.

<sup>4</sup> An account of the fairly extensive literature in this respect is given by G. Seidlitz, 'Beiträge zur Descendenztheorie,' pp. 31—36, Leipzig, 1876, and supplemented by Hering, "Ueb. d. Beweg. d. sternförm. Pigmentzellen, &c.," communicated by von Hoyer, 'Centralbl. f. d. med. Wiss.,' 1869, No. 4, p. 49.

cetes,<sup>1</sup> by Haeckel in *Protomyxa*,<sup>2</sup> in the ciliated epithelium of calcareous Sponges,<sup>3</sup> the segmentation spheres of Siphonophora,<sup>4</sup> and by Clark among Flagellata.<sup>5</sup>

Protoplasmic movement, properly so called, is to be distinguished from the changes of external form and internal arrangement of the protoplasm which occur during growth and reparation, segmentation, fertilization, &c., of cells, principally by its greater rapidity and its non-relation to all growth and reproduction. But here, again, there is no sharp limit, as shown, for instance, in the phenomena of fission among *Protamœbæ*, *Amœbæ*, and colourless blood-corpuscles, and the increasing internal movements preparatory to spore formation, which occur in the cells of many *Algæ* and *Fungi*.<sup>6</sup>

Historical.—The oldest description of a protoplasmic movement which I have been able to come across is by Rösels von Rosenhof.<sup>7</sup>

The small “*Proteus*” which this excellent observer described and figured in the year 1755 was a large freshwater *Amœba*. Rösels distinguished between a richly granular interior and a hyaline peripheral portion (“*ein zartes äusseres Häutlein*”), figured the unceasing changes of shape, the assumption of the spherical form in consequence of mechanical excitation, and the phenomenon of fission; this latter, by-the-by, the first direct observation of a cell-division. Following close upon this, twenty years later, comes Bonaventura Corti’s<sup>8</sup> celebrated

<sup>1</sup> ‘*Zeitschr. f. wissensch. Zool.*,’ x, p. 153, 1860.

<sup>2</sup> ‘*Jenaische Ztschr.*,’ iv, p. 87, 1868.

<sup>3</sup> *Ibid*, v, p. 543, 1870.

<sup>4</sup> ‘*Entwicklungsgeschichte d. Siphonoph.*,’ pl. vi, fig. 36; pl. xiv, fig. 93, Utrecht, 1869.

<sup>5</sup> ‘*Mem. Bost. Soc. Nat. Hist.*,’ 1867, pls. ix and x.

<sup>6</sup> Concerning the latter phenomena cf. W. Hofmeister, ‘*Die Lehre von der Pflanzenzelle*,’ p. 45, &c., Leipzig, 1867.

<sup>7</sup> Rösels von Rosenhof, ‘*Der monatlich herausgegebenen Insectenbelustigungen*,’ dritter Theil, pp. 621—623, pl. ci, figs. A—W., Nürnberg, 1755. The “*Proteus*” described shortly previous to this by Baker, is *Trachelocerca* olor, a holotrichous Infusorian.

<sup>8</sup> B. Corti, ‘*Osservazioni microsc. sulla Tremella e sulla circolazione del fluido in una pianta acquajola*,’ Lucca, 1774.



description of the rotation of the "cell-sap" in Characeæ. The wide distribution of the phenomenon in vegetable cells was specially demonstrated in the first third of this century through the observations of Meyen ('*Vallisneria*, *Hydrocharis*,' 1827), Robert Brown ('*Staminal Hairs of Tradescantia*,' 1831), Amici, and others. Of the highest importance was Dujardin's<sup>1</sup> description of a shapeless contractile body-substance in many lower organisms (*Rhizopods*, *Infusoria*, *Polyps*, &c.). His observations upon this substance, which he called *Sarcode*, and its movements are even to-day of actual importance. He was the first to observe the granular streaming in the pseudopodia of *Rhizopods*. Soon phenomena of movement were discovered in the cells of higher animals which resembled those of *sarcode* in a remarkable way (*Limax* eggs, Dujardin 1837; *Planarian* eggs, v. Siebold 1841; colourless blood-corpuscles, Wharton Jones 1846; and others). Ecker<sup>2</sup> showed (1849) the connection between the various "organised" (muscles, ciliary hairs) and the "unorganised" contractile substances of animals, and a year later (1850) Ferd. Cohn<sup>3</sup> expressed his opinion, and supported it with good reasons, that the actively motile substance of vegetable cells, which since 1846 (H. von Mohl<sup>4</sup>) has been distinguished as protoplasm from cell-sap, "and the contractile substance and *sarcode* of the zoologists, if not identical, were in the highest degree analogous structures."

That it was not, as had till then under the influence of the older cell-theory been supposed, the cell membrane which effected the contractions of animal cells, but the so-called cell contents was proved by Donders.<sup>5</sup>

<sup>1</sup> Dujardin, "Observ. Nouv. sur les *Cephalopodes* microscop," 'Bull. de la Soc. des Sc. natur. de France,' No. 3, 1835; 'Ann. Sci. Nat.' iii, 2nd sér., p. 312, 1835; Ibid. iv. p. 343, 1835 (*Sarcode*).

<sup>2</sup> Alex. Ecker, "Zur Lehre vom Bau und Leben der contractilen Substanz der niedersten Thiere," 'Ztschr. f. wis. Zool.' i, pp. 218—249, 1849.

<sup>3</sup> F. Cohn, "Nachträge zur Naturgeschichte des *Protococcus pluvialis*," 'Nova Acta Acad. Leop. Cæs., &c.' xxii, 2, p. 605, 1850.

<sup>4</sup> H. von Mohl, "Ueber die Saftbewegung im innern der Zelle," 'Bot. Zeitung,' p. 73, 1846.

<sup>5</sup> F. C. Donders, "Form, Mischung und Funktion der elementaren Gewebs-

The actual identity of animal and vegetable protoplasmic movement has been since then more closely proved through the morphological and physiological investigations of Max Schultze,<sup>1</sup> Unger,<sup>2</sup> De Bary,<sup>3</sup> Haeckel,<sup>4</sup> and Kühne;<sup>5</sup> and a more complete knowledge of the movement and its manifold conditions has been afforded by these authors as well as by Naegeli, Brücke, Heidenhain, Cienkowski, Hofmeister, and others. The wandering of amœboid cells in animal tissues, brought into general notice by von Recklinghausen<sup>6</sup> (1863), and the importance of this for many physiological and pathological events in animal organisms was shown by this author, and by Stricker, Cohnheim, and others.

## II. PHYSICAL AND CHEMICAL PROPERTIES OF CONTRACTILE PROTOPLASM.

Contractile protoplasm appears optically as a homogeneous, transparent, almost always colourless mass, with a higher theile in Zusammenhang mit ihrer Genese betrachtet," 'Ztschr. f. wis. Zool,' iv, p. 249, 1852 (translated from the 'Nederl. Lancet,' Derde, ser. i, pp. 84, et seq., 1851-52).

<sup>1</sup> Max Schultze, 'Ueber den organismus der Polyophthalmien,' Leipzig, 1854. "Ueber innere Bewegungserscheinungen bei Diatomeen," 'Arch. f. Anat. u. Physiol.,' 1858, p. 330. "Ueber Muskelkörperchen und das was man einer Zelle zu nennen habe," 'Arch. f. Anat. u. Physiol.' 1861, p. 1 (in this article the term protoplasm is transferred to the "contents" of animal cells). 'Das Protoplasma der Rhizopoden und der Pflanzenzellen,' Leipzig, 1863.

<sup>2</sup> F. Unger, 'Anatomie und Physiologie der Pflanzen,' pp. 273-284, Pesth, Wien und Leipzig, 1855.

<sup>3</sup> A. de Bary, "Die Mycetozen," 'Ztschr. f. wis. Zool.,' x, pp. 88-175 (especially p. 121, et seq.), 1859, pls. vi-x. The second, considerably enlarged, edition separately under the title 'Die Mycetozen' (Schleimpilze) Leipzig, 1864.

<sup>4</sup> E. H. Haeckel, 'Die Radiolarien,' Berlin, 1862 (specially p. 89, et seq.), "Ueber den Sarkodekörper der Rhizopoden," 'Ztschr. f. wis. Zool.,' xv, p. 342, 1865, and many other memoirs, especially in the 'Jenaische Zeitschrift.'

<sup>5</sup> W. Kühne, 'Untersuchungen über das Protoplasma und die Contractilität,' Leipzig, 1864; also 'Arch. f. Anat. and Physiol.,' 1859, pp. 564, 748.

<sup>6</sup> F. von Recklinghausen, "Ueber Eiter- und Bindegewebskörperchen," 'Arch. f. Path. Anat.,' xxviii, p. 157, pl. ii, 1863.

refractive index than water but lower than oil. In some cases, as, for instance, where it has the form of thick fibres or skin-like layers with a prevailing movement in one direction (pseudopodia of *Actinosphœrium Eichornii*, cortical protoplasm of *Stentor*), it is distinctly doubly refracting, and, indeed, as in the case of muscles and cilia, with a single positive axis, the optical axis coinciding with the direction of the movement.<sup>1</sup>

Different portions of one and the same protoplasmic mass may have different refractive powers. In the case of naked amœboid protoplasm the more superficial layers are more highly refractive than the deeper: in the pseudopodia of *Actinosphœrium* and many *Rhizopods* a strongly refracting axial layer may be distinguished from the remainder. During the movements the refractive power of the same portion usually changes to a considerable extent.

With regard to its mechanical properties, protoplasm may present a greater or less degree of fluidity, does not mix with water, and is capable of swelling up; it presents great cohesive power, great extensile power, trifling elasticity and a tendency to take on the form of droplets. These properties vary, however, not only for different varieties of protoplasm, but at different spots of the same protoplasmic mass, and often differ even in the same spots within short intervals of time. With naked amœboid protoplasm the superficial layer is firmer than the central mass and may even permanently or temporarily pass into a strong membrane. As a general rule, no such membrane exists so that solid particles can be taken in by the outer layer of the body at any chosen spot, as may be easily observed by feeding with coloured particles (indigo, carmine, &c.).<sup>2</sup> In many cases the central mass is the firmer, the superficial portion being very soft and often quite sticky (pseudopodia of many *Rhizopods*, *Actinosphœrium*, &c.).

Protoplasm almost without exception contains certain bodies

<sup>1</sup> 'Arch. f. d. Ges. Physiol.,' xi, p. 449 and 454, &c., 1875.

<sup>2</sup> E. Haeckel, 'Die Radiolarien,' pp. 104—106, v. Recklinghausen, 'Arch. f. pathol. Anat.,' xxviii, p. 184; W. Preyer, *ibid*, xxx, p. 420; M. Schultze, 'Arch. f. mikroskop. Anat.,' i, p. 23.

which play a passive rôle with regard to the movement. Setting aside as of casual occurrence solid particles which have been taken in from without and nuclear structures, they—namely, the granules and vacuoles contained in the protoplasm—are generally of exceedingly minute size. The granules may be very numerous, but on the other hand they may be of very sparse occurrence. The majority of the granules are albuminous, some are of a fatty nature while others are inorganic (e.g. carbonate of lime, in certain *Myxoplasmodia*). Occasionally coloured particles are present (many *Myxomycetes*, *Protamoeba aurantiaca*, &c.).

Very commonly the granules occur exclusively in the central portions of the protoplasm. In this case a fairly thick glass-like outer layer or skin devoid of granules may be distinguished from a granular and therefore opaque central mass (this is specially distinct in *Amoebæ* and *Myxoplasmodia*). These two may appear to be very sharply separated from one another during actual movement, although they are continually becoming mixed and separated again.

Where the granular protoplasm becomes drawn out into very thin threads (pseudopodia of *Rhizopods*, *Radiolaria*, &c., the thread-like networks of *Noctiluca*, numerous vegetable cells) the granules often project beyond the superficial layer. Indeed, in such cases they often exist chiefly in the superficial layer. Foreign particles moreover easily get stuck to the outer layers of naked protoplasm, and are then moved along in the same way as the true granules (*Rhizopods*, *Oscillatoria*, *Diatoms*, &c.).

The densely granular portions of the protoplasm appear as a rule to possess less cohesion than those which are devoid of granules. The granular central mass of *Myxoplasmodia* and *Amoebæ* often flows within the firmer superficial layer like a fluid emulsion in a bladder. Not unfrequently the granules exhibit irregular shaking, dancing movements, apparently quite similar to those exhibited by the smallest particles suspended in a thin fluid (Brownian molecular movement). This is the case for instance in the endoplasm of *Vorticellæ*, in the interior



of many Myxomycetes, and in the protoplasm of numerous vegetable cells. Special vacuoles filled with fluid in which such movements take place are by no means always or even frequently to be discovered. The whole plasma appears rather to have little more cohesion than a thin fluid at such spots. At the surface of very thin protoplasmic threads the extent to which the granules move is greater than in the more hyaline axis. Such fibres, moreover, very easily flow together, forming "sheets" like ordinary mucous threads, while the hyaline layer surrounding protoplasmic masses does not readily do so.

That the flowing together does not, or at least does not always, depend only upon the prior cohesive power of the substance is well shown in that the pseudopodia of different individual Rhizopods,<sup>1</sup> as well as the processes of plasmodia belonging to different species<sup>2</sup> never fuse with one another.

Doubtless the above-cited differences in cohesive power depend essentially upon the differences in the amount of imbibition-water, which is shown by the refractive index varying in direct proportion to the amount of cohesion. These differences in cohesive power are also artificially produced along with corresponding changes of volume and refracting power by means which cause swelling or shrinking. In mobile granular protoplasm a separation of the fluid in the form of small droplets frequently takes place—vacuole-formation. The protoplasm may in consequence acquire a frothy appearance.

In resting protoplasm the form of the vacuoles is for the most part perfectly globular. During movement they may be much drawn out, but always return to their globular form.

This also holds good for the form of the gas-bubbles<sup>3</sup> which have been observed in some instances in protoplasm.

<sup>1</sup> Max Schultze, 'Das Protoplasma der Rhizopoden und der Pflanzenzellen,' p. 25, 1863.

<sup>2</sup> Cienkowski, "Zur Entwicklungsgeschichte der Myxomyceten," 'Jahrb. f. wissensch. Bot.,' iii, p. 335, 1863; de Bary, 'Die Mycetozoen,' 2 Aufl., p. 40, 1864.

<sup>3</sup> Th. W. Engelmann, "Beiträge z. Physiol. d. Protoplasma," 'Arch. f. d. ges. Physiol.,' ii, p. 307, 1869; 'Zool. Anz.,' i, p. 152, 1878.

For our knowledge of the chemical composition of pure protoplasm we are really dependent upon its micro-chemical reactions. On this account our existing knowledge in this respect is extremely scanty. It must be specially noted that there is no chemical mark by which contractile can be separated from non-contractile protoplasm.

In life the reaction of protoplasm is generally weakly alkaline or neutral;<sup>1</sup> in *Æthaliium septicum* it is always distinctly alkaline.<sup>2</sup> Now and then I have seen blue litmus particles change within a few minutes after being taken into the contractile endoplasm of *Stylonychia mytilus* and *S. pustuluta*, *Paramœcium aurelia*, and *Amœba diffluens*, to a red colour and remain so.<sup>3</sup>

Among the solid substances which often make up together 10—20 per cent. of the total weight, albuminous granules make up by far the greater mass, as is usual in protoplasm. And, indeed, albuminous granules are always observable, at any rate at a lower temperature than that at which they coagulate (generally under 50°). In addition to these, carbohydrates (in the plasmodium of *Æthaliium* there is a quantity of glycogenous substance<sup>4</sup>), fat, inorganic substances, especially potash compounds, are seldom absent. Lecithin is also frequently present. In the plasmodium of *Æthaliium septicum* a peptic enzym is found.<sup>5</sup>

<sup>1</sup> Th. W. Engelmann, "Ueber die Flimmerbewegung," 'Jenaische Zeitsch. f. Med. u. Naturw.,' iv, p. 469, Anm., 1868.

<sup>2</sup> 'Briefl. Mittheil.,' von De Bary; C. W. F. Krukenberg, 'Unters. d. physiol. Instit. d. Univ.,' Heidelberg, ii, p. 273, 1878.

<sup>3</sup> This is possibly due to an acid secreted in an attempt to digest the particles. I have observed Vorticellæ in a watery solution of aniline blue taking it in rapidly and becoming filled with blue vacuoles, the contents of which are gradually absorbed, the blue colouring matter reappearing of a different tint in the contractile (excretory) vacuole.—TRANSE.

<sup>4</sup> 'Briefl. Mittheil.,' von W. Kuhne.

<sup>5</sup> Krukenberg and others.

### III. THE SPONTANEOUS MOVEMENTS OF PROTOPLASM.

In accordance with the peculiarities mentioned in the introduction, protoplasmic movements in general present a great variety and change in the manner of their appearance, and this is the case to such an extent that it is impossible to give a short description applicable to all cases. Certain definite types may, however, be distinguished, and we may almost limit ourselves to describing these and referring to the fact that they are connected with each other by numerous transitional forms.

#### 1. Movements of Naked Protoplasm.

Three chief types may be distinguished, and may be called respectively—Amœboid movement, Streaming movement, and Gliding movement.

The amœboid movement shows itself in the protrusion and retraction of smooth, round, conical, or flattened, at first generally hyaline processes, into which the granular mass from the interior streams in and out.<sup>1</sup> The processes may remain separate from one another, or they may ramify and even form networks. In the simplest cases of this kind of movement only slow unimportant changes of the external form of the mass occur, which do not cause a change of position. This is the case with the egg cells of many Vertebrata before fertilization. The phenomena soon become more complicated, as shown in the movements of Amœbæ, Myxamœbæ, Arcellæ, Difflugia, many Monads, numerous egg cells (Hydra, Sponges), the white blood-corpuscles of most animals, pus-corpuscles, wandering cells in connective tissue, and many epithelia (frog's cornea), &c.

We now come to those extensive and often very active streamings which occur in very granular central masses, and those considerable changes of shape which come about

<sup>1</sup> First, and very well described by O. Fr. Müller, 'Animalcula infusoria, &c.,' p. 10, 1786.

in consequence of the appearance and disappearance of processes which, although taking on very various forms, almost always remain unfused with one another. As these processes fasten themselves to fixed bodies, they can, by shortening themselves, draw the remaining protoplasm after them, and produce a movement of translation. The rapidity with which they can do this varies with the particular body and its surroundings, but always remains microscopic. A velocity of 0.5 mm. a minute which is sometimes attained by an *Amœba* may be considered as exceptionally rapid. The force with which amoeboid movement takes place may be regarded as of a quite important value. The wandering cells of the frog's cornea, for instance, move between the fibrillæ and lamellæ and between the other epithelium cells, which in doing so they must push apart from one another.

Fig. 1 shows the different forms (*a—p*) which the same

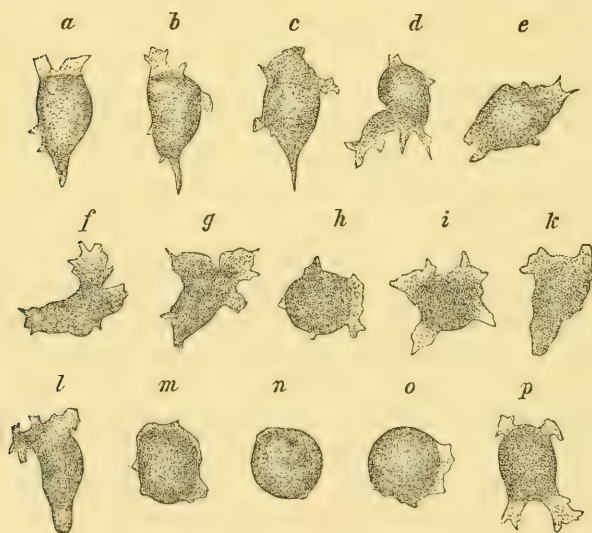


FIG. 1.—A colourless blood-corpuscle of a frog under the influence of (*a—m*) a gradually increasing temperature, which (*n—p*) subsequently diminishes.



colourless blood-corpuscle of a frog assumed at intervals of one minute.

The plasmodium of *Myxomycetes* is very good for the study of amœboid movement, on account of the great size of the protoplasmic masses and the extreme rapidity of the movements, which are visible even to the naked eye. The processes here run together in networks, which may cover more than four square centimetres.

A. De Bary<sup>1</sup> describes the movements thus:—"There are two varieties. In the first place, in every mass of transparent plasmodium a large number of granules are to be seen in active streaming. In all the thread-shaped branches there is always one current only along the axis of the branch; where branching takes place the current divides, following the branches, or if the movement is taking place in the other direction the currents in the branches flow together into the main stream; not unfrequently, however, the streaming proceeds in one lateral branch only, while in the other no movement, or one in the opposite direction, prevails.

"In smooth, skin-like expanses numerous branching streams generally run either in the same or in different directions, and often streams going in directly opposite directions run side by side.

"The peripheral substance, within which the granulated protoplasm streams, exhibits a movement which appears for the most part unconnected with this, and which consists in a slow flowing or undulating change of the margin, small processes being continually extruded and withdrawn again. The granules are often quite unaffected by these movements, but sometimes are carried in smaller or larger numbers into the small tentacle-like branches. The activity of the peripheral movements varies much, under continued observation. Very trifling changes in the margin are to be seen, and the flat expanses in particular often look like a perfectly still surface sprinkled with motionless granules while the streams flow through it.

<sup>1</sup> A. De Bary, 'Die Mycetozen,' 2 Aufl., p. 43, et seq., Leipzig, 1864.

"Especially in the latter case the plasmodium has often the appearance of being composed of two quite different substances—a streaming fluid filled with granules, and a viscous, slowly-flowing portion, the former appearing to move within the latter in special canals with firm walls. But new streams may be often seen to arise in the transparent portion of the plasmodium, and the granules in a resting portion suddenly fall into a main stream; others, on the contrary, cease moving, and completely take on all the properties of portions which are at rest. The resting granules on the margin of a strong stream can suddenly fall into movement, following the stream, and all sharp line between streaming and resting portions vanish.

"If one observes streams coming from the extremities of branches one of two different phenomena may be seen. On the one hand, the extremities, very much drawn inwards, pass into a condition of energetic contraction, and the streaming is most active near the extremities and diminishes in rapidity in a centrifugal direction (towards the periphery). On the other hand, the extremities from which the stream comes may sink slowly together, and the rapidity of the stream increase steadily in a centrifugal direction.

"Where an active stream runs into the ends of branches, and these rapidly swell up and new branches are given off, it looks as though the granular mass were forcibly pressed into the ends. At the same time, it is generally very obvious that the stream going towards the ends of the branches increases in velocity in a centrifugal direction" (pp. 47—48).

According to Hofmeister,<sup>1</sup> the granular stream of Myxomycetes commences at the periphery and extends backwards spreading as it does so; this may be also stated for numerous cases of amoeboid movement and is of theoretical value.

The streaming movement occurs in almost all Rhizopods, in Heliozoa and Radiolaria, and in some Monads. Out of the protoplasmic body spring long thin threads of protoplasm—pseudopodia (root-feet). And as a rule upon the surface there are a great number of fine granules in most active streaming

<sup>1</sup> W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 17, Leipzig, 1867.

movement. The threads themselves often at times show no movement, and at other times only slow changes of form which consist in lengthenings and shortenings, in the formation of nodules, also of curved or irregular bends and branchings. They may be quite withdrawn into the protoplasmic mass. When they touch each other they fuse together, very easily forming sheets.

The characteristic phenomenon of the movements of granules is described by Max Schultze<sup>1</sup> in the following way:—"It is a gliding, a flowing of the embedded granules in the substance of the threads. With greater or less rapidity these granules move in the threads either towards the periphery or in the opposite direction, and often, even in the thinnest fibres, in both directions at once. They may either simply pass one another or may move round one another, and after a short pause move on in the original direction, or the one granule may carry the other along with it. Like pedestrians in a broad street, the granules may swarm together in a broad fibre, many of them stopping from time to time and shaking or trembling merely, always, however, following a direction corresponding with the long axis of the threads. Often they stand still in the midst of their course and reverse their direction, the most (? Engelmann), however, succeed in reaching the extremity of their threads and here first change their direction.

"All the granules in a thread do not move with the same rapidity, so that one often overtakes another, the more rapidly moving granules pushing the others on, or, the latter stopping the former in their course. Where threads join one another, granules often pass over from one into the other, and at such places there are often broad expanses which have been formed out of agglomerations of thread substance out of which then, as independent processes, further threads are formed, or into which, already existing ones are, as it were, absorbed. Many granules quite evidently run in the most superficial layers of the threads from which they may be seen projecting. Possibly they all have a superficial position. Besides the small granules

<sup>1</sup> Max Schultze, 'Das Protoplasma,' &c., p. 11.

there are often larger lumps of substance like spindle-shaped swellings, or lateral prominences of a thread moving in the same way as the granules. Foreign bodies, which have got stuck to the thread substance and taken in, partake also of the movement.

“The greatest rapidity of single granules hitherto observed (Schultze in *Miliola*) attains 0.02 mm. in a second. Generally it is considerably less. It is extremely sluggish for instance in sun animalcules.”

Gliding movement.<sup>1</sup>—The peculiarity of this case is that on the outside of a firm cell integument, extremely thin layers of protoplasm devoid of granules move along, and by means of their movement the whole body progresses upon a firm basis in a gliding or creeping manner; relatively solid bodies which remain sticking to this layer can be moved onwards along its upper surface. The direction of the movement is generally straight forward (Diatoms) or spiral (*Oscillatoria*), sometimes backwards sometimes forwards. The rapidity seldom exceeds 0.04 mm. in a second. It is almost continually changing in the same individual. The force with which the movement takes place may attain a considerable amount. This type of movement is exhibited by most *Bacillaria* (rocking movement of Diatoms) and *Oscillatoria* as well as by young stages of *Nostocaceæ* and *Rivulariæ*.

The superficial protoplasm of these organisms is during life it appears, never visible, on account of its extreme thinness and low refractive power. Its presence was formerly inferred only, on account of the movement produced.<sup>2</sup> In many cases it is only rendered visible by means which produce coagulation.<sup>3</sup>

<sup>1</sup> Many botanists, following Nägeli's example, use this term to express the streaming of granules on the surface of threads of protoplasm.

<sup>2</sup> Max Schultze, “Ueber die Bewegungen d. Diatomeen,” ‘Arch. f. Mikr. Anat.’ i, pp. 376—402, pl. xxiii, 1865.

<sup>3</sup> Th. W. Engelmann, ‘Ueber die Bewegungen der Oscillarien und Diatomeen,’ ‘Arch. f. d. ges. Physiol.’ xix, p. 8, 1878.



## 2. Movements of Protoplasm bounded by firm Integuments.

This case is chiefly realised in vegetable cells. We can with the botanists distinguish two chief varieties :—Circulation and Rotation.

Circulation.— Here contractile protoplasmic threads stretch inwards from the cell wall, traversing the cell space, which is filled with fluid ; they vary in number and are continually changing their position, form, and size. The direction and rapidity of their movement are generally inconstant, being



FIG. 2.—Cells from the staminal hairs of *Tradescantia* (after Kühne).  
*A*. Fresh, in water. *B*. The same cell after slight local electrical stimulation. *a—b*. The region stimulated. *c*. Clumps and nobs of contracted protoplasm.

often quite different in immediately neighbouring spots. The threads can divide, fuse, and form sheets, and generally exhibit streaming granules, and behave on the whole like the pseudopodia of Rhizopods (see above). This type occurs in numerous vegetable cells, in hairs from plants (*Cucurbita*, staminal hairs from *Tradescantia*, fig. 2, &c.). It also occurs in *Noctiluca*, *Dicyema* (Entoderm cells), in the cartilaginous cells of the tentacles in *Medusæ* and in the gill-fibres of *Branchiomma*, in *enchondroma*-cells, &c.

**Rotation.**—The protoplasm lining the walls of a cell (the outermost pellicle excepted) rotates as a connected mass around the interior of the cell, generally following constant tracks and with an even velocity. The direction of the movement is always one almost parallel to the long axis of the cell. Any protoplasmic contents which may exist—nuclei, chlorophyll granules, crystals—rotate along with it, generally without changing to any considerable extent their relative position.

The best known instances of this movement are the cells of the *Characeæ*, the leaf-cells of *Vallisneria spiralis* and *Ceratophyllum submersum*, and the root hairs of *Hydrocharis morsus-ranæ*.

Here must also be classed the rotation of the endoplasm of *Paramœcium bursaria* and *P. aurelia* and some other Infusoria (e.g. *Vorticellæ*).

#### IV. GENERAL CONDITIONS OF SPONTANEOUS PROTOPLASMIC MOVEMENT.<sup>1</sup>

##### 1. Temperature.

For every contractile protoplasm there is a lower and a higher temperature at which its spontaneous movements stop, directly and under all circumstances. The minimum generally lies at about 0° and the maximum about 40° C. Within these two temperatures is the province of manifest contractility; and the

<sup>1</sup> Most important literature:—Dutrochet, 'Compt. rend.,' ii, pp. 775—784, 1837 (*Chara*); Max Schultze, 'Das Protoplasma der Rhizopoden, &c.,' 1863; W. Kühne, 'Unters. über das Protoplasma, &c.,' 1864.

velocity of the movement increases, as a rule, with the temperature, and, indeed, in all special cases a definite constant velocity corresponds to a definite degree of temperature. This, however, is no longer the case if shortly before, a rapid and extensive fluctuation of temperature has taken place. Such fluctuation acts like a mechanical or electrical excitation, and will be spoken of later. For similar increments of temperature, the increase of velocity appears in many cases to be greater, the higher the absolute temperature.

Naegeli,<sup>1</sup> in the terminal cell of *Nitella syncarpa*, which was slowly warmed under the microscope, observed that a distance of 0.1 mm. was traversed by the rotating layer in 60 sec. at 1° C., in 24 sec. at 5° C., in 8 sec. at 10° C., in 3.6 sec. at 20° C., in 1.5 sec. at 31° C., in 0.6 sec. at 37° C. Schultze<sup>2</sup> has shown, on the contrary, that the granular streaming in *Miliola*, which at ordinary temperatures is already very rapid—0.2 mm. in a second—cannot be accelerated by further warming.

There is apparently in every case a certain higher limit of temperature at which the movement attains its highest velocity. This optimum temperature generally lies several degrees below the maximum temperature compatible with movement. If the temperature rises above the optimum the movement indeed becomes just at first even more active, but dies out after some time. There is generally some delay before this occurs, but this is the less the nearer the prevailing temperature to the maximum. If the temperature attains the maximum, all movement stops in a moment. The protoplasm then enters upon a condition of apparent death or rigor—temporary heat rigor, heat tetanus—in which it remains, as though under continued artificial stimulation. It is drawn together so as to expose the smallest surface, and is only relaxed when cooling occurs, when, and in which case only, the movements are able to start once more. The optical properties of the protoplasm are not necessarily altered in this condition.

<sup>1</sup> C. Naegeli, 'Beiträge zur wissenschaft. Botanik,' 2 Heft, p. 77, Leipzig, 1860.

<sup>2</sup> Max Schultze, 'Das Protoplasma, &c.,' p. 47.

Naked masses of protoplasm of microscopic size, such as *Amœbæ*, colourless blood-corpuscles, when gradually warmed up to the maximum, become spherical. Protoplasmic threads of *Rhizopods*, vegetable cells, &c., generally become at first varicose, and finally withdrawn into the main mass of the protoplasm. Fig. 3 shows the different shapes assumed by a

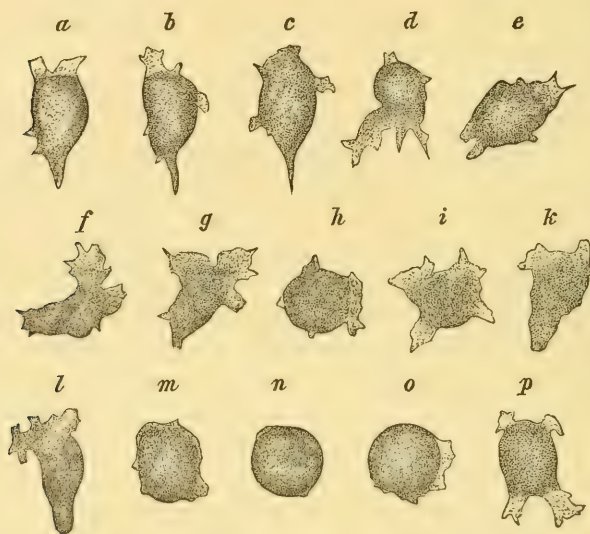


FIG. 3.—A colourless blood-corpuscle of a frog under the influence of (*a—m*) a gradually increasing temperature, which (*n—p*) subsequently diminishes.

colourless blood-corpuscle of a frog, under the influence of gradually warming and subsequent cooling, at intervals of five minutes.

From *a* to *c* the temperature was  $12^{\circ}$  C., the shape is only slightly changed. At *c* the microscope, with the preparation in a moist chamber, was placed in a Sach's warm box filled with water at  $50^{\circ}$  C. After a few minutes the movements had become visibly more active, and the cell crept forwards with, up to *l*, an ever-increasing rapidity. At *m* it commenced, and at *n* had completely entered into heat-rigor. The preparation



was then removed from the warm chamber and cooled gradually to the temperature of the room, 12° C. At *o* changes of shape commenced again, and at *p* were already quite active.

When the temperature exceeds the maximum the protoplasm dies at a certain degree, which may be called the ultramaximum, and exhibits suddenly a shrinking and loss of clearness, on account of the coagulation of the albumens, and, as a rule, the vacuoles are extruded. This heat-death or lasting heat-rigor may also come about even at a lower temperature when the warming has been very long continued. Subsequent cooling then naturally produces no further change.

Freshwater *Amœbæ* which, after warming for one minute at 35°, showed only passing rigor, Kühne found would, after fifteen minutes' warming at the same temperature, become completely globular and lose all power of movement. After shorter warming of *Amœbæ* at 40° they appeared like "globular, sharp, and doubly-contoured vesicles, which contained a brownish-looking and, by transmitted light, very opaque lump, which was generally attached firmly to one side of the periphery, and filled up some three parts of the spherical space. The remaining space was filled with a transparent clear fluid, in which small granules in active molecular movement swarmed about." Kühne subsequently treated such individuals with water at 45°; the molecular movement in the previously clear portion of the vesicle stopped, and formed here also a firm coagulum.<sup>1</sup>

When the protoplasm enters suddenly into heat-rigor it has no time to change its form. Max Schultze<sup>2</sup> saw, for instance, the protoplasmic threads of *Miliola*, when warmed quickly, at any rate up to 45°, enter at once into rigor: similarly with *Tradescantia*.

Concerning the value of the maximum and ultramaximum for different species of contractile protoplasm, the following table gives some idea:

<sup>1</sup> W. Kühne, 'Untersuchungen über das Protoplasma,' pp. 43—45.

<sup>2</sup> M. Schultze, 'Das Protoplasma, &c.,' p. 22.

|                                       | Maximum about | Ultramaximum about      |              |
|---------------------------------------|---------------|-------------------------|--------------|
| <i>Didymium serpula</i> . . .         | 30° C. . . .  | 35° C.                  | Kühne.       |
| <i>Aethalium septicum</i> . . .       | 39° C. . . .  | 40° C.                  | „            |
| <i>Actinosphærium Eichornii</i> . . . | 38° C. . . .  | 43° C.                  | M. Schultze. |
| <i>Miliola</i> . . . . .              | 38° C. . . .  | 43°—48° C.              | „            |
| <i>Urtica urens</i> . . . . .         | 44° C. . . .  | 47°—48° C.              | „            |
| <i>Tradescantia virginica</i> . . .   | 46° C. . . .  | 47°—48° C.              | „            |
| <i>Vallisneria spiralis</i> . . .     | 40° C. . . .  | 47°—48° C. <sup>1</sup> |              |
| <i>Nitella syncarpa</i> . . . .       | 37° C. . . .  | —                       | <sup>2</sup> |
| <i>Chara flexilis</i> . . . . .       | — . . . .     | 45° C. <sup>3</sup>     |              |

If the temperature sinks gradually to the minimum, the spontaneous movements stop after they have first become slower and slower. Simplifications of the shape generally occur, and existing processes and branchings are gradually absorbed and new ones are no longer developed. But the complex form may remain, under certain circumstances, as observed by Kühne in *Amœba diffluens* (l. c., p. 46) and *Actinosphærium* (l. c., p. 68). Optical changes do not generally accompany the entrance into this cold rigor. But Hofmeister<sup>4</sup> saw the walls of a protoplasmic mass in *Cucurbita*, after standing for a long time at 0° C., become filled with vacuoles and quite frothy. Artificial excitation remains potent, and raising the temperature above the minimum causes a resumption of the movements.

It appears that contractile protoplasm may be kept at the minimum temperature, and even much below it, for an almost unlimited time without sustaining permanent injury. No lower temperature limit, ultraminimum, at which death inevitably ensues, has been described. Even after actual freezing the protoplasm when thawed will, under certain circumstances, resume its spontaneous contractions. And in this case it does not even appear necessary that the thawing should take place very slowly, a condition which otherwise is very essential for the revivification of organic structures rich in water.

<sup>1</sup> Jürgensen, 'Stud. d. Physiol. Inst.,' i, p. 104, Breslau, 1861; Schultze, &c.

<sup>2</sup> Naegeli, 'Beitr. z. wissensch. Bot.,' ii, p. 77.

<sup>3</sup> Dutrochet, 'Compt. rend.,' 1837, ii, p. 775.

<sup>4</sup> W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 55.

Kühne<sup>1</sup> allowed *Tradescantia* hairs to freeze to the walls of a platinum vessel without adding water. It was quickly lowered to a temperature of  $-14^{\circ}$  C., and allowed to remain for more than five minutes at this temperature without death of the protoplasm ensuing. Taken out and quickly examined in water, no trace of protoplasmic network was present, but the violet interspaces in the cells presented near the naked nucleus a great number of separated round droplets and little lumps. A few seconds later these began to exhibit exceptionally active amœboid movement. After some minutes they began to run together into single larger droplets, and these, again, united with other groups, and in about ten minutes the original protoplasmic network was there again; and even after twenty-four hours the threads were found in active streaming. Hofmeister<sup>2</sup> has corroborated these observations.

## 2. Imbibition Water.

The amount of imbibition-water acts like the degree of temperature. For every protoplasm there is a maximum and minimum for the amount of contained imbibition water at which spontaneous movements stop. Exact determinations have not been made, but the minimum may be stated as on the average below 60 per cent. and the maximum over 90 per cent. Within these limits the activity of the protoplasm generally varies, with a corresponding increase of volume and decrease of refractive index, with amount of the contained imbibition water. Rapid changes in the extent of concentration of the medium, which induce rapid swelling or more especially shrinking, may act like an excitation (see below). There is always an optimum for the amount of imbibition water.

When the maximum is gradually approached the protoplasm takes on its simplest form (globules, varicosities, &c.). Removal of the excess of water with indifferent substances (weak sugar solutions, salt solutions, &c.) often reinduce the move-

<sup>1</sup> W. Kühne, 'Unters. über das Protoplasma,' U. S. W., p. 100, et seq.

<sup>2</sup> W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 54.

ments after even some minutes of water-rigor. When treated for any long period with distilled water the protoplasm dies. The phenomena attending this are not always the same. The protoplasm may form vacuoles and deliquesce, or it coagulates at once, in which case the form corresponding to the non-contracted condition may remain preserved for a considerable time longer.

The withdrawing of water by indifferent or diluted solutions leads eventually to a temporary or lasting rigor (dry-rigor). In vegetable cells, moreover, as discovered by Al. Braun<sup>1</sup> in *Chara*, the protoplasm generally withdraws itself from the cell-wall as a continuous sac, the movements continuing for a considerable time. Naked protoplasm (*Amœbæ*, *Myxomycetes*) which has been shrunk up by the action of 1—2 per cent. salt solution often becomes covered with a large number of fine, pointed, hyaline, cilia-like processes. After dilution with water the protoplasm returns to its original condition.<sup>2</sup> Protoplasm which has been completely dried in the air at ordinary temperatures and entered in consequence into rigor, can under certain circumstances after mixture with water become active again, and this even after several years. This is certainly the case for instance with encysted *Amœbæ* and *Infusoria*. It may, however, be also observed in naked plasmidia and in many other even quite highly organised bodies.

It is important to note that when the concentration is exceptionally slowly increased, protoplasm can in many cases (all ?) accommodate itself to the solutions, while if the action is more

<sup>1</sup> Al. Braun, 'Monatsber. d. Berliner Acad.,' 1852, p. 225; further, W. Hofmeister, loc. cit., p. 52.

<sup>2</sup> Kühne, 'Untersuchungen, &c.,' pp. 48, 82; cf. De Bary, 'Die Mycetozoen,' 2 Aufl., p. 46, pl. ii, fig. 16; iii, figs. 11 and 12; Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 24, fig. 28; V. Czerny, "Einige Beob. über Amœben," 'Arch. f. mikr. Anat.,' v, p. 159, 1869; Strasburger, "Studien über das Protoplasma," 'Jen. Zeitschr. f. Naturwissensch.,' x, p. 407, Jena, 1876. The cilia-like processes are produced, according to most of these observers, without any noteworthy change in the concentration of the surrounding medium, in connection with a shrinking in of a process; this I can also corroborate.



rapid it would be at once destroyed. It appears that in such a case no corresponding strong contraction takes place.

In sea-water which I had preserved for more than a year, and which by gradual evaporation had become so concentrated that there was 10 per cent. of saline matter, numerous Protozoa, even Worms, Arthropods, Diatoms, Green Algæ, &c.,<sup>1</sup> still lived and apparently quite comfortably. I was also able to repeat Czerny's<sup>2</sup> experiment with fresh-water *Amœbæ*, and accustom them in the course of several weeks to 4 per cent. solutions of common salt. With the action of 10 per cent. solution of common salt, according to Kühne,<sup>3</sup> fresh-water *Amœbæ* immediately pass into spherical bodies which quickly break up and throw out a network of fine mucous-like threads, while the rest passes into the form of coarser and finer particles which move about with active molecular movement. Salt-water *Amœbæ* behave in a similar manner.

### 3. Oxygen.

In media quite free from oxygen spontaneous movement can without the least doubt only go on for a short time—at most for some hours. The gradually advancing cessation can always in its early stages be stayed by the admission of oxygen, and indeed in this way only. With regard to the connection between the energy of the movement and the amount of the absorption of oxygen in the surrounding media, only so much can be said with certainty, that the movement in many (all?) cases is a permanently maximal one at very slight pressures, far under the normal. At great pressures of oxygen (3—6 atmospheres) it diminishes, but is accelerated when the pressure is lowered again.

Evidently the living protoplasm enters into chemical union with the surrounding media, and the oxygen thus firmly com-

<sup>1</sup> Cf. also Dutrochet, 'Compt. rend.,' 1837, ii, pp. 781, 782.

<sup>2</sup> V. Czerny, 'Arch. f. mikr. Anat.,' v, p. 158, et seq., 1869.

<sup>3</sup> Kühne, 'Unters. über das Protoplasma, &c.,' p. 48; cf. also Czerny and others.

bined, of which under normal circumstances a certain amount must be taken into each protoplasmic body, is constantly used up during the movements, probably by the giving off of  $\text{CO}_2$ .

Corti observed the streaming cease in the cells of *Chara*, when the air was removed by olive oil, as well as after long standing under the best vacuum obtainable under the receiver of an air-pump. Hofmeister<sup>1</sup> observed a cessation in *Nitella* after five minutes in olive oil, and after thirteen minutes in very rarified air. In the first case the movement started again after the restoration of the air in thirty minutes, and in the second case after twenty-two minutes.

Kühne removed the atmospheric air by means of pure hydrogen. After the gas had been passed for more than twenty-four minutes, fresh-water *Amœbæ* fell motionless to the bottom of the drop (they responded in this condition to induction shocks, but a markedly stronger excitation was necessary in this case). Spontaneous movements recommenced seventy-five minutes after the entrance of air. Plasmodia of *Myxomycetes* and also the protoplasm of *Tradescantia* hairs showed no further movement, after some hours of contact with hydrogen, and were in active movement again a few minutes after the readmission of air. And even after standing twenty-four hours in hydrogen readmission of air reinduced the movements. I found that contractile cells from the lymph sac of a frog required two hours' transmission of the purest hydrogen through a hermetically sealed moist chamber to stop the movements: most of the cells became spherical. The same happened with fresh-water *Amœbæ*. A drop of hæmoglobin solution after being under similar conditions for ten minutes, no longer showed in a microspectroscope the two absorption bands of oxy-hæmoglobin, which were previously very distinct. Under very long treatment with pure hydrogen protoplasm dies completely, generally first becoming cloudy and forming vacuoles and finally falling to pieces.

Tarchanoff,<sup>2</sup> following in the steps of Paul Bert's discovery

<sup>1</sup> W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 49.

<sup>2</sup> Tarchanoff, 'Arbeiten der St. Petersburger Gesellsch. d. Naturf.,' vii,

of the injurious action towards life of oxygen at high pressures, experimented with this agent. He observed that the colourless blood-corpuscles of the frog become round and motionless at 3—6 atmospheres of oxygen pressure, and resume their movements at ordinary pressures.

#### 4. Other Chemical Conditions—Poisons.

Like all the vital phenomena of elementary organisms the spontaneous movements of the protoplasm can only continue for any time when the reaction of the imbibition fluid is neutral or almost neutral. A trifling excess of alkali or even more surely of acid causes a cessation which for a time may be counteracted by neutralization of the excess.

Dutrochet<sup>1</sup> saw the movement in *Chara* stop completely in potash solution of 0.05 per cent. in thirty-five minutes, in potash or soda of 0.1 per cent. in two or three minutes, in tartaric acid of 10 per cent. in ten minutes, of 0.1 per cent. in an hour.

Max Schultze confirmed the injurious action of diluted acids (hydrochloric, acetic, and osmic) and alkalis on *Miliola*,<sup>2</sup> *Actinosphærium*,<sup>3</sup> *Tradescantia*,<sup>4</sup> *Myxomycetes*. And Kühne also on *Amœba*,<sup>5</sup> *Actinosphærium*,<sup>6</sup> *Myxomycetes*,<sup>7</sup> *Tradescantia*.<sup>8</sup>

In diluted caustic alkali the protoplasm swells up strongly, deliquesces and completely breaks up. Before the cessation

p. 122, 1876 (Russian). I am acquainted only with Hoyer and Mayzel's abstract in Hofmann and Schwalbe's 'Jahresber.,' v, 1876, p. 22.

<sup>1</sup> Dutrochet, 'Compt. rend.,' 1837, ii, p. 781.

<sup>2</sup> Max Schultze, 'Das Protoplasma, &c.,' pp. 22 and 37.

<sup>3</sup> Ibid., p. 32.

<sup>4</sup> Ibid., p. 42.

<sup>5</sup> W. Kühne, 'Untersuch. über das Protoplasma und die Contractilität.,' p. 49 (HCE 0.1 per cent., KHO 0.1 per cent., and 1 per cent.).

<sup>6</sup> Ibid., p. 64 (HCE 0.1 per cent., KHO 0.1 per cent., Ammonia Vapour); p. 67 (CO<sub>2</sub>).

<sup>7</sup> Ibid., p. 85 (Ammonia Vapour); p. 89 (CO<sub>2</sub>).

<sup>8</sup> Ibid., p. 100 (HCE, KHO).

the normal movement is often observed to be accelerated.<sup>1</sup> In diluted acids death generally sets in with opacity and shrinking (coagulation of the albumen).<sup>2</sup> Carbonic acid has also this result, if passed over the preparations in a concentrated stream for some time.<sup>3</sup> Movements, which have stopped in consequence of the action of weak CO<sub>2</sub> can by its replacement by air, generally also by hydrogen, be reinduced, in consequence of which any existing opacity is removed.

Ether or chloroform act in the same way as CO<sub>2</sub> causing a temporary or permanent coagulation. They need only be mixed in small quantities with the air to effect their destructive action, which is also at first easily stayed by the introduction of pure air.<sup>4</sup>

It is interesting to note, as they specially agree in this respect with the contractile substance of muscle fibres, that many kinds of protoplasm are acted on in a poisonous way by veratrin.<sup>5</sup> Kühne observed that freshwater *Amœbæ*,<sup>6</sup> *Actinosphærium*,<sup>7</sup> and *Myxomycetes*,<sup>8</sup> in exceptionally diluted, barely alkaline, even neutral solutions of this poison, become quickly coagulated, becoming opaque and completely breaking up. *Tradescantia* cells, however, still showed the normal movements after seventeen hours' immersion in a watery veratrin solution.

Binz<sup>9</sup> and others have observed that quinine exercises a strongly destructive action on many kinds of protoplasm and on colourless blood-corpuscles. On the other hand, I have given frogs such large doses of quinine sulphate by subcuta-

<sup>1</sup> By Dutrochet (l. c., p. 781), in *Chara*; By Kühne (l. c., p. 49), in *Amœba*.

<sup>2</sup> Kühne, l. c., pp. 49, 64.

<sup>3</sup> Ibid., p. 51 (*Amœba*); p. 67 (*Actinosphærium*); p. 90 (*Myxomycetes*); p. 106 (*Tradescantia*).

<sup>4</sup> Ibid., p. 66 (*Actinosphærium*); p. 100 (*Tradescantia*).

<sup>5</sup> Kühne, l. c., p. 47, et seq.

<sup>6</sup> Kühne, l. c., p. 47.

<sup>7</sup> Ibid., p. 65.

<sup>8</sup> Ibid., p. 86, et seq.

<sup>9</sup> C. Binz, "Ueber die Einwirkung des Chinin auf Protoplasma-bewegung," 'Arch. f. Mikr. Anat.,' iii, p. 383, 1867.



neous injection as to kill them, and have observed the lymph-corpuscles after some hours in active movement.

#### V. BEHAVIOUR OF PROTOPLASM TOWARDS ARTIFICIAL STIMULATION.

Protoplasmic contractions may, like the movements of muscles and other excitable organs, be called forth, not only by the normal physiological stimulus, but by numerous external influences, so-called artificial stimuli, or, as is more frequently the case, existing movements can be thus influenced. These stimuli are in reality the same as those for other excitable structures; as a general rule, any disturbance of the electrical equilibrium acts as a stimulus when it takes place with a certain rapidity or exceeds a certain amount, also, all electrical impulse, change of temperature, mechanical or chemical influence.

The amount of irritability, as measured by the weakest stimulus which calls forth an effect, varies with the kind of the protoplasm, the kind of stimulus, and the special conditions.

The protoplasm of fresh-water *Amœbæ*, *Diatoms*, *Valisneria*, &c., behaves to very weak induction currents in the same manner as do blood-corpuscles.<sup>1</sup>

The protoplasm of *Pelomyxa*, which otherwise is not specially sensitive, is violently affected by suddenly admitted strong light, which has not necessarily any stimulating effect upon other kinds of protoplasm.<sup>2</sup>

The general conditions which affect artificial stimulation, with which it increases or diminishes, are the same as those which affect the power of spontaneous movement. They have, however, rather wider limits, as is shown, amongst other things, by the fact that artificial stimuli are potent even after spontaneous movements have ceased to exist (e.g. as a result of cooling, warming, withdrawal of oxygen, excess of  $\text{CO}_2$ ).<sup>3</sup>

<sup>1</sup> Author's observations.

<sup>2</sup> Th. W. Engelmann, "Ueber Reizung contractilen Protoplasmas durch plötzliche Beleuchtung," 'Arch f. d. ges. Physiol.' xix, p. 1, 1878.

<sup>3</sup> Cf. Kühne, 'Unters. über das Protoplasma, &c.,' pp. 45, 53 (*Amœbæ*), p. 106 (*Tradescantia*).

The visible phenomena resulting from artificial stimulation may vary to a very great extent. We must here consider especially whether the protoplasm was in movement at the time of the excitation or not, and in the former case the kind and energy of the movement; further, whether the protoplasm was excited at the same time all over and to the same extent, more especially whether the stimulation was stronger at some spots than at others; further, whether it was free to move or was shut in a firm cell-wall, &c. The following special observations give some idea of the manifold character of the phenomena.

Generally, the effect of artificial stimulation shows itself in the directly excited portion of the protoplasm receding without visible change of volume, drawing itself together so as to expose the least possible external surface, and taking on a spherical form, just like a stimulated muscle. The rapidity and power with which it does this generally range within the same limits which exist for the spontaneous movements of the same object.

### 1. Electrical Stimuli.

Electrical currents only cause a movement in protoplasm when they flow directly through it, never from a distance only.<sup>1</sup> Moreover, it is especially sudden changes in the intensity of a current which are followed by movements. The change in the current, as in the case of muscles, is not nearly so important as is the occurrence itself of the current, but after the breaking of a constant current, responsive movement takes place, as a rule, only in those cases where the current, after attaining its full force, has continued to flow for some time.<sup>2</sup>

This time may amount, as in the case of *Amœba*, to more than a second. Kühne<sup>3</sup> observed in *Actinosphærium* that

<sup>1</sup> Becquerel ('Compt. rend.,' 1837, ii, p. 786) found strong galvanic streams (10—30 elements) quite inactive when passed through a wire bound round *Chara*, whatever the direction of the current.

<sup>2</sup> Th. W. Engelmann, "Beiträge zur allgem. Muskel und Nervenphysiologie," 'Arch. f. d. ges. Physiol.,' iii, pp. 311 and 312, 1870.

<sup>3</sup> Kühne, 'Unters. über das Protoplasma,' p. 59, et seq.

the effect remained at the side of the animal which was in contact with the positive pole so long as the circuit remained closed. As a rule, however, the protoplasm soon regains its original condition so long as the current continues to flow with a constant intensity. Becquerel<sup>1</sup> has observed this in Chara.

Making a constant current is a specifically stronger stimulus than breaking it, but the latter requires a longer duration of the current, or that the current should be more intense. Often, more especially when the object is very sensitive to the making shock, breaking a very strong current is quite inactive.<sup>2</sup>

The effect called forth increases very markedly within certain limits with the suddenness and the extent of the variation of the intensity of the current. Induction shocks are therefore as a rule more effective than making constant currents.

The effect of stimuli following one another at short intervals may be cumulative, and in this way singly ineffective stimuli may together produce a visible and powerful effect. The intervals between the stimuli, in order that the summation of effects may take place, must be as a rule of considerable amount (e. g. with many *Amœbæ* and vegetable cells four seconds and more), and it appears that the more sluggish the spontaneous movements of an object the longer are the intervals necessary.<sup>3</sup>

After powerful excitations exhaustion sets in, stronger excitations are then necessary to produce the same effect, or, the excitations remaining the same, longer intervals of time for recovery. Very strong excitations will kill protoplasm; it becomes opaque, enters into rigor, and shrinks up or breaks to pieces: or they call forth secondary effects which completely upset the course of physiological events.

Behaviour of various types of protoplasm towards electrical stimulus.—The action of single induction shocks

<sup>1</sup> Becquerel, 'Compt. rend.,' 1837, ii, p. 787.

<sup>2</sup> 'Arch. f. d. ges. Physiol.,' iii, p. 311, 1870.

<sup>3</sup> Author's observations.

upon frogs' blood-corpuscles in active amœboid movement, according to Golubew,<sup>1</sup> is that after some time (generally  $\frac{1}{4}$ —1 min.) the processes at first pointed become blunted, and gradually draw themselves into the cell-body. "If the stimulus acts more strongly a very rapid and complete drawing together of the cell so as to form a rounded lump may be observed. In this condition the cell remains for some time, and then resumes its ordinary movements." With still stronger excitation the cell almost always becomes suddenly spherical. In a few minutes a small droplet suddenly extrudes from some spot, increases in size for a time, more body substance flowing into it; it then decreases in size again, while at one or more spots new droplets are extruded. In this manner changes of form may occur which are at first very rapid and striking, but soon become slower; the droplets are drawn in, and the ordinary irregularly pointed processes are reformed. Fresh-water Amœbæ behave in a similar manner, only much more quickly.<sup>2</sup> It must be noted that after a latent period, which with weak excitation amounts to some seconds but with stronger excitation may become imperceptibly short, there follows a slowing, or more probably a stopping altogether of the granular streaming movements, and of the shifting of position. After this follows—it is apparently simultaneous only when the excitation is very strong—a withdrawal, a shortening and thickening of the processes, which may amount within a few seconds to an assumption of a spherical shape. Soon after this are thrown out, generally by jerks, one or more hyaline protuberances into which the granules proceed forthwith to stream. The latter fasten themselves often to the surface of the outer pellicle. One of the processes enlarges more and more, stretches itself lengthwise, and finally takes the whole of the mass of the protoplasm

<sup>1</sup> A. Golubew, 'Ueber d. Erschein., welche electricische Schläge an den Sog. farblosen Bestandtheilen des Blutes hervorbringen,' 'Sitzungsber. d. Wiener Acad.,' lvii, p. 557, et seq., 1868.

<sup>2</sup> Th. W. Engelmann, "Beitr. zur Physiol. d. Protoplasma," 'Arch. f. d. ges. Physiol.,' ii, p. 312, et seq., 1869.



into itself. After as much as ten seconds' stimulation *Amœbæ* will recover their original appearance and movement. *Myxomycetes* appears to behave in essentially the same way. The phenomena are, however, somewhat altered here, as the size of the object admits, as a rule, of partial excitation only.<sup>1</sup>

Rhizopods (*Miliola*, *Actinosphærium*) draw their pseudopodia in when electrically excited, and these as a rule shortly become varicose.<sup>2</sup>

A stronger excitation is required to excite the pseudopodia which lie at right angles to the direction of the current than for those which lie parallel to it. The protoplasmic threads of vegetable cells which exhibit circulation behave in a similar manner (*Tradescantia* type).<sup>3</sup>

So long as the excitation is weak, as with amœboid protoplasm, slowing and stopping of the movements generally occurs at first, then varicosities, lumps, &c. are formed. Especially instructive are the phenomena in the case of partial excitation. Kühne<sup>4</sup> observed (cp. fig. 2, p. 402) with *Tradescantia*, "that at one portion of the cell the stronger threads drew together, forming lumps and spheres, in which, after a resting period, movement of the granules commenced, which might have been mistaken for molecular movement if it had not been evident that the granules obeyed another impulse, owing to the very altered condition of the ground substance. As soon as the lumps and spheres became flattened again they moved onwards one by one with the streams in the neighbourhood which had

<sup>1</sup> Kühne, 'Unters. üb. d. Protoplasma, &c.,' p. 75, et seq.

<sup>2</sup> M. Schultze, 'Das Protoplasma, &c.,' p. 38; Kühne, 'Unters., &c.,' p. 56.

<sup>3</sup> M. Schultze, 'Das Protoplasma, &c.,' p. 43, et seq. Heidenhain, "Notizen über die Bewegungserscheinungen, welche das Protoplasma in den Pflanzenzellen zeigt.," 'Stud. d. physiol. Institut. zu Breslau,' 2 Heft, p. 66, 1863.

<sup>4</sup> Kühne, 'Unters. über das Protoplasma, &c.,' p. 99 (*Tradescantia*). W. Velten, "Einwirkung strömender Elektrizität auf die Bewegung des Protoplasma, &c.," 'Sitzber. d. Wiener-Mathem. Naturw.,' Cl. lxxiii, p. 351, et seq., 1876. The phenomena of swelling described by Velten have not been observed by other observers, nor by myself. I must state that, at any rate, when the current is not over maximal, they do not take place in any marked manner.

continued to flow, and finally came to completely resemble them. Where the lumps are formed the finer threads become broken through, and in such places the thickenings are quickly drawn out again into threads either from one or both sides."



FIG. 4.—Cells from the staminal hairs of *Tradescantia* (after Kühne.)  
*A.* Fresh, in water. *B.* The same cell after slight local electrical stimulation. *a—b.* The region stimulated. *c.* Clumps and nobs of contracted protoplasm.

The rotating protoplasm of *Chara* and *Vallisneria* cells, when excited simultaneously at all points, shows a slowing or stopping of the movement.<sup>1</sup>

After this, if the excitation is sufficient, it draws itself up

<sup>1</sup> Becquerel, 'Compt. rend.,' ii, p. 787, 1837. Jürgensen, 'Stud. d. physiol. Instit. zu Breslau,' 1 Heft, p. 99, 1861. Velten, 'Sitzber. d. Wiener Mathem.-Physiol. Cl.,' lxxiii, p. 350, et seq., 1876.

together, as appears to be almost universally the case with vegetable cells containing moving protoplasm, at the short transverse walls (ends of the cells), and the whole mass contracts, so as to expose the least superficies, the process thus corresponding with the formation of spherical bodies by naked protoplasm when subjected to excitation. Brücke<sup>1</sup> observed upon the stinging hairs of *Urtica* that after short powerful excitations threads of protoplasm with pointed ends and swellings in the middle, or knob-like, club-shaped processes stood out from the primordial utricle, being afterwards withdrawn again.

## 2. Thermal Stimuli.

Both positive and negative alterations of temperature may act as stimuli, and produce results similar or identical with those produced by electrical stimuli. This, of course, only when the alterations fall within the temperature range of contractility. In this case also the effect is the more pronounced and lasting the more rapid and extensive the change of temperature. Negative changes (always?) appear to act to a specifically greater extent than positive.

If after a change the temperature remains constant, the movements gradually assume the condition which they would have assumed by quite gradual warming or cooling, as the case might be, to a corresponding degree.

In *Chara*, which exhibited a moderately rapid rotation in water at 7°, Dutrochet<sup>2</sup> observed that the movements completely ceased in four to five minutes when plunged into water at 32°. After some hours in water at 32°, the movements commenced again, and in two more hours had become quite active once more. Replaced in water at 7° the movements were again destroyed in four minutes, but recommenced quite slowly after half an hour at this temperature. After somewhat slower

<sup>1</sup> E. Brücke, "Das Verhalten der sog. Protoplasma-ströme in den Brennhaaren von *Urtica urens*," 'Sitzber. d. Wiener Akad.,' xlv, p. 2, 1863.  
M. Schultze, 'Das Protoplasma, &c.,' p. 45.

<sup>2</sup> Dutrochet, 'Compt. rend.,' ii, p. 777, 1837.

warming, from  $18^{\circ}$  to  $27^{\circ}$ ; from  $27^{\circ}$  to  $34^{\circ}$ , and from  $34^{\circ}$  to  $40^{\circ}$ , the rotation stopped for a time, varying from some minutes to an hour.

Hofmeister<sup>1</sup> observed a temporary cessation of the rotation in a preparation of *Nitella* which was taken from a room at a temperature of  $18.5^{\circ}$  C. into one at  $5^{\circ}$  C. and left there for two minutes. The same thing<sup>2</sup> happened after six to eight minutes with hairs of *Ecbalium agreste*, which exhibiting active streaming at  $16-17^{\circ}$  C., were taken into a temperature of  $40^{\circ}$  C., the protoplasmic network becoming much simpler and quite motionless. The streaming commenced again at the latter temperature only after a pause of from half an hour to two hours, and, once started, attained in a few minutes the activity normal to such a temperature. Rapid cooling from  $40^{\circ}$  to  $17^{\circ}$  C. rendered the protoplasm of the same object motionless. Again, "at numerous spots it had formed knotty varicosities." The movements recommenced only after seven minutes, and became once more normal (temperature constant at  $16^{\circ}$  C.) only after eighteen minutes.<sup>3</sup> On rapidly warming the stinging hairs of *Urtica* up to  $40^{\circ}$  C. and higher, Schultze<sup>4</sup> often observed the same curious changes of form of the protoplasm which Brücke has described as occurring after strong shocks with the magneto-electro-motor. (See above.) Kühne's and Hofmeister's observations upon the effect of rapidly freezing *Tradescantia* cells have already been described.

### 3. Light Stimuli.

The greater number of kinds of contractile protoplasm appear insensible to light or changes of light. This is the case, for instance, in the protoplasm of the colourless blood-

<sup>1</sup> W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 53; cp. also Hugo de Vries, in 'Flora,' 1873, p. 25 (*Hydrocharis morsus ranæ*).

<sup>2</sup> Ibid., p. 55.

<sup>3</sup> Ibid., p. 54.

<sup>4</sup> M. Schultze, 'Das Protoplasma, &c.,' p. 48.



corpuscles and other amœboid cells of Vertebrata and Invertebrata,<sup>1</sup> the common Amœbæ, many Rhizopods, Infusoria, and vegetable cells.<sup>2</sup> In green cells from which light is shut off, the movement stops, but only with the whole vegetation of the plant; in *Chara*, for instance, as shown by Dutrochet,<sup>3</sup> only after twenty-four to twenty-six days.

In special cases the intimate structure of the protoplasm obviously alters with long exposure to light or the reverse. The plasmodium of *Æthodium* creeping upon the surface of tan while in the dark will withdraw itself again into the deeper layers upon exposure to bright light, and while in the light it puts forth only short much crowded processes, in the dark long, narrow, thin branches are developed.<sup>4</sup> Here then illumination appears to act similarly to artificial stimuli. It is also well known that changes in the illumination cause changes in the contractile pigment cells which occur in the skin of many Fishes, Amphibia, and Reptiles—changes which bring about a change in the colour of the whole animal. The black pigment cells, for instance, in the frog's cutis, which in the dark are much branched, contract gradually upon exposure to a bright light to small spheres, in consequence of which the skin appears lighter in colour. But it appears that in this case the action of the light upon the contractile elements is indirect, and is transmitted

<sup>1</sup> Author's and other observations.

<sup>2</sup> Cp. Jul. Sachs, "Ueber den Einfluss des Tageslichtes, &c.," 'Botan. Ztg.,' 1863. W. Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 49, 1867. G. Kraus, "Ueber Versuche mit Pflanzen in farbigem Lichte," 'Sitzungsber. d. naturf. Gesellsch.,' Halle, v, 20 May, 1876; 'Botan. Ztg.,' 1876, p. 504 (yellow light is without influence upon *Hydrocharis*, *Trianea*, *Chara*, *Vallisneria*, *Elodea*, *Pilobolus*, *Urtica dioica*, *Navicula*).

<sup>3</sup> Dutrochet, 'Compt. rend.,' ii, p. 779, 1837.

<sup>4</sup> Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 21, 1867; Baranetsky, "Influence de la lumière sur les plasmodia des Myxomycetes," 'Mém. Soc. des Sciences Nat. Cherbourg,' xix, p. 321, 1875 (finds the blue rays specially active, the yellow not).

by the nerves, as Lister<sup>1</sup> and Pouchet<sup>2</sup> have specially shown with respect to the reflex of the eye.

Differing, however, from the above-cited cases, we have the behaviour of the protoplasm of *Pelomyxa palustris*.<sup>3</sup>

This large fresh-water *Amœba* creeps about very actively in the dark, but when suddenly exposed to the light (diffused daylight is sufficient) the streaming of the granules ceases, and in a few seconds it draws itself together into a sphere. If it continues exposed to the light, movements recommence, but of a weak sluggish nature only. If the darkness is changed quite gradually to light (the change extending over about a quarter of an hour), there is no excitation-effect. There is also no excitation-effect when, after long illumination, the light is suddenly shut off.

It should here be noted that when portions of green leaves of *Phanerogams*, Mosses, or Fern prothalli are put into strong shade and kept there for some time, they assume a darker colouring in consequence of the gradual change of position of the chlorophyll grains which are contained in the protoplasm. These heap themselves together under the influence of light—especially of short-wave rays—at the sides of the cells which are turned towards the surface of the leaf, while in the dark they accumulate at the walls of the cells perpendicular to the surface. Although these changes certainly depend upon the movement of the protoplasm, it must remain uncertain how far they express a direct influence of light on protoplasm, or an indirect influence in consequence of the primary changes which the light exerts upon the chlorophyll bodies.<sup>4</sup>

<sup>1</sup> Jos. Lister, "On the Cutaneous Pigment System of the Frog," 'Phil. Trans. Roy. Soc.,' cxlviii, p. 627, 1859.

<sup>2</sup> G. Pouchet, "Sur les rapides changements de coloration provoqués expérimentalement chez les poissons," 'Compt. rend.,' lxxxii, p. 866, 1871. Cp. also G. Seidlitz, 'Beiträge zur Descendenztheorie,' Leipzig, 1876, who has collected the instances of colour changes among animals.

<sup>3</sup> Th. W. Engelmann, "Ueber Reizung contr. Protopl. durch plötzliche Beleuchtung," 'Arch. f. d. ges. Physiol.,' xix, p. 1.

<sup>4</sup> Cp. J. Böhm, 'Sitzgsber. d. Wiener Acad.,' xxii, p. 476, 1856; xxxvii, p. 475, 1859; xlvii, p. 352, 1863. A. Famintzin, 'Jahrb. f. wissensch. Bot.,'

## 4. Mechanical Stimuli.

All sudden mechanical disturbances of any importance act as mechanical stimuli—pressing, pulling, bruising, and tearing to pieces. The phenomena are really the same as those called forth by electrical excitation.

More than one hundred years ago Rösel<sup>1</sup> observed that when touched *Amœbæ* draw themselves together. Colourless blood corpuscles, *Rhizopods*, &c., after violent pressure draw in their processes and often become varicose. The streaming of the plasmodia of *Myxomycetes* is easily slowed or brought to a standstill for some time by any disturbance whatsoever.<sup>2</sup> The protoplasmic threads in the staminal hairs of *Tradescantia* will, after strong sudden bruising, tear apart, “draw themselves together into short clubs or balls, and fuse partly with the protoplasm, which is heaped up around the nucleus, and partly with the protoplasm lining the cell-wall”—the primordial utricle. After ten to fifteen minutes the normal arrangement and movement commences again.<sup>3</sup> The rotation in *Chara* cells, as was observed by Gozzi,<sup>4</sup> and afterwards by Dutrochet,<sup>5</sup> and others, stops after the tying of a ligature round the cell or sharply bending. But rotation soon commences again in the two halves. Dutrochet<sup>6</sup> observed a stoppage of some minutes after cutting or tearing the cell. It is without doubt due to mechanical excitation that freshly-made preparations of *Chara*, *Vallisneria*, &c., only exhibit motionless protoplasm. The movement recommences

vi, p. 1, 1867. Borodin, ‘Mél. Biol. Petersb.,’ vi, 1867; vii, 1869. Frank, ‘Jahrb. f. wissensch. Bot.,’ viii, p. 216, 1871. ‘Botan. Ztg.,’ 1871, No. 14 and 15. Jul. Sachs, ‘Ber. d. sächs. Ges. d. Wiss. Mathem.-phys. Cl.,’ xxii, 1859; ‘Lehrb. d. Bot.,’ 4 Aufl., p. 722, 1874.

<sup>1</sup> Rösel von Rosenhof, ‘Der monatl. herausgeg. Insectenbelustigungen, dritter Theil, p. 621, Nürnberg, 1755.

<sup>2</sup> H. de Bary, ‘Die Mycetozoen,’ 2 Aufl., p. 49; Hofmeister, ‘Lehre von der Pflanzenzelle,’ p. 26.

<sup>3</sup> Hofmeister, loc. cit., p. 50.

<sup>4</sup> Gozzi, in ‘Brugnatelli Giornale de fisica,’ 2 Dec., p. 199, 1818.

<sup>5</sup> Dutrochet, ‘Compt. rend.,’ ii, p. 780, 1837.

<sup>6</sup> Dutrochet, ‘Compt. rend.,’ ii, p. 780, 1837.

after the preparation has been left quiet for a time.<sup>1</sup> This also holds good according to some observations, for Diatoms and Oscillatoria.

### 5. Chemical Stimuli.

Sudden chemical reactions produce the same effects as are observed after electrical excitations, but complicated and interrupting accessory phenomena are often present (shrinking, swelling, coagulation, &c.). So only in very few cases is it possible to observe the special effects of the excitation as evinced by the form and temporary structure.

Sudden changes in the amount of the contained water may act as stimuli.

Dutrochet<sup>2</sup> placed *Chara* in salt solution of more than 1 per cent. After four minutes the movements stopped, and after eight minutes more recommenced again. Gradually this became very rapid, and continued for ten days. A similar preparation in which the movement had been stopped, and after ten hours' immersion had become quite active again, was plunged into pure water at the same temperature, and this caused a cessation of the movement in four minutes which lasted for five minutes. Hofmeister<sup>3</sup> says, after observations on *Chara*, *Vallisneria*, *Hydrocharis*, and *Tradescantia*:—"The treatment of a cell which contains streaming protoplasm with a watery solution of a substance not directly injurious to the vital activity of the plant when of a concentration sufficient to cause the sudden drawing together of the protoplasmic contents of the cell, stops all flowing movement of the protoplasm for a short time during the contraction of the protoplasmic contents, but a rapid streaming of the undivided peripheral layer soon commences again."

After the sudden dilution of a solution in which a cell with easily permeable walls (e.g. leaf-cell of *Vallisneria*, root

<sup>1</sup> Hofmeister, l. c., p. 50.

<sup>2</sup> Dutrochet, 'Compt. rend.', ii, pp. 781, 782, 1837.

<sup>3</sup> Hofmeister, loc. cit., p. 52; cp. also *ibid.*, p. 27, for corresponding observations on *Didymene serpula*.



hairs of *Hydrocharis*), is showing the normal movement, a temporary cessation only, takes place.<sup>1</sup>

Fresh-water *Amœbæ* which I accustomed to salt water of 2·5 per cent. drew themselves rapidly together on the addition of  $\frac{1}{2}$  per cent. salt solution, but recommenced, however, to move in the original manner after a few minutes. Czerny<sup>2</sup> has made similar observations.

Similar observations have also been made concerning the influence of acids and alkalies.

In Dutrochet's<sup>3</sup> experiments on *Chara* an immersion for five minutes in potash or soda of 0·05 per cent., or tartaric acid of 0·1 per cent., caused an acceleration of the movement.

Kühne<sup>4</sup> found that after very short and weak applications of ammonia vapour to *Actinosphærium* the processes became very small, short, and strongly varicose, but after a long pause resumed their original condition.

## VI. THEORETICAL CONCLUSIONS.

No theory of protoplasmic movements, leading back to their elementary physical and chemical processes, can be deduced from the hitherto collected facts. As it is, in fact, possible only to directly perceive the mechanical portion of the process, any theoretical conclusion must be limited to what relates to the mechanism of the movements, and an investigation into the nature and connection which the visible phenomena have with the invisible molecular action which underlies them must be left to futurity.

Every attempt at an explanation of the mechanism of protoplasmic movements must not only take into account all the known modifications of such movements, as Hofmeister<sup>5</sup> has

<sup>1</sup> Hofmeister, loc. cit., p. 53.

<sup>2</sup> V. Czerny, "Einige Beobachtungen über Amöben," 'Arch. f. Mikr. Anat.,' v, p. 158, 1869.

<sup>3</sup> Dutrochet, 'Compt. rend.,' ii, p. 78, 1837.

<sup>4</sup> Kühne, l. c., pp. 64 and 65; pp. 48 and 49 (*Amœba*); p. 82 (*Myxomycetes*); also Schultze, l. c., p. 32 (*Actinosphærium*); and p. 37 (*Miliola*).

<sup>5</sup> Hofmeister, 'Die Lehre von der Pflanzenzelle,' p. 59.

already rightly insisted, but must be applicable in its principle to all other phenomena of contractility. The essential agreement which exists between them all in the mode of appearance and conditions of occurrence, and especially the gradual transition between them, show that we have here in all cases to do with expressions of the same principle, with the same elementary mechanism of movement. As a starting-point for the closer analysis of protoplasmic movement, we may take the acknowledged fact that each smallest microscopically distinguishable particle of every contractile protoplasmic mass is capable of independent movements. Ample proof of this is furnished by the changes of form and position which may take place, spontaneously or in consequence of artificial excitation, at every point in any otherwise quiescent protoplasmic mass, as also in the smallest artificially isolated protoplasmic particles.

It follows as a very close and, I believe, most natural consequence, that we may regard protoplasm as an aggregate of most minute contractile, excitable form-elements, and that the movement as a whole is the result of changes of form of these very small elements. The nature and cause of the changes of form of the latter remain provisionally undetermined.

We have, as yet, no reason for considering that the most minute particles of protoplasm which are to be distinguished with the microscope are the contractile elements themselves; we must think of the latter as still smaller—as of molecular dimensions. With regard to their form, we may take it for granted that when in a condition of maximal excitation they are almost spherical, or as nearly spherical as possible, and when not excited are generally elongated, with a fibre-like shape. The reason for the first of these assumptions lies in the observation that even the smallest particle of protoplasm which can be experimented upon assumes a spherical shape as a result of artificial excitation, that is to say, if it be not already spherical. Supporting the second assumption we have, firstly, the fact that the smallest protoplasmic particles which have contracted to spherical shape in consequence of excitation,

when that excitation is removed generally take on an elongated, even exceptionally slender, form, fibres, pseudopodia, &c.; secondly; resting hyaline protoplasm, as already described, not unfrequently split up completely into exceptionally fine fibrillæ; thirdly, the smallest distinguishable form-elements of other contractile structures (ciliated organs, myophanes, muscle-fibres) have in a resting stage an elongated form.

The mechanical behaviour of naked protoplasm especially teaches us that the changes of form, particularly the shortening of its contractile elements, must take place with a force which, as a rule, exceeds at any rate the force which the elements, if they were fluid, would put forth in order to assume a spherical form.

For shortness the hypothetical contractile elements will be called in the sequel "Inotagmata." In connection with this it must be pointed out that in them the power is generated which causes the contraction, and which has been described as molecular combination (Tagmata, Pfeffer<sup>1</sup>). Very probably all inotagmata are positive uniaxial doubly-refracting, hence contractility in general appears to be bound up with the existence of positively uniaxial particles.<sup>2</sup>

The active as well as the passive phenomena of protoplasmic movements compel us further to the assumption that the inotagmata of the protoplasm are not, like those of muscles and ciliary processes, arranged together in a relatively firm manner, with the axes all in one definite direction, but are, as a rule, fastened together very loosely, and are capable of moving one against the other in all directions, as a natural consequence of which the possibility of the temporary or permanent grouping of a lesser or greater number of inotagmata into definitely-shaped larger masses (fibres, membranes, &c.) is not excluded.

As a reason for the possibility of alteration of arrangement of the protoplasmic particles, and in connection with the prevailing views concerning the molecular structure of organised masses, we must assume the existence of a capability for the

<sup>1</sup> W. Pfeffer, 'Osmotische Untersuchungen,' p. 32, Leipzig, 1877.

<sup>2</sup> "Contractilität und Doppelbrechung," 'Arch. f. d. ges. Physiol.,' xi, 1875.

imbibition of important quantities of water between inotagmata and inotagma groups. The motility, as already shown, increases or diminishes with the quantity of this water.

The preceding observations afford a first step only towards explaining protoplasmic movement, in so far as they allow the many different forms under which it appears and the changes which it undergoes as a result of all kinds of influences, to be referred to a single process, which itself requires further explanation—the changes of form of inotagmata.

Let us detail at least some of the most important cases in this connection.

### 1. Formation of Spheres by Naked Protoplasm on Excitation.

This clearly must follow from the simultaneous assumption of a spherical form by inotagmata, in so far as therewith the surface attraction which they exert over one another, and thus the cohesion of the entire mass, must be equally observable everywhere and in all directions.

A good proof of the correctness of the latter conclusion exists in the sudden assumption of a spherical form by the air-bubbles in the protoplasm of *Arcella* upon electrical excitation. As in so doing the volume of the air-bubbles undergoes hardly any diminution, it is evident that the assumption of the spherical form cannot be a consequence merely of a contraction of the peripheral layer of the protoplasm, as is often thought. The force with which this approximation to the spherical form is brought about depends essentially upon the force with which the inotagmata change their form, and upon the average amount of the cohesion of the protoplasm; and as the latter decreases as the amount of imbibition water increases, the force must, as a general rule, decrease as the amount of the water increases. As a matter of fact, in very thin fluid protoplasm, e.g. many plasmodia, the force of gravity is sufficient to prevent the drawing together to form a sphere.

The formation of varicosities, the retraction or fusion with one another of fibre-like or flattened processes (pseudopodia



and such like), is easily explained from the above-stated principles.<sup>1</sup>

## 2. Origin of Processes.

If in a protoplasmic mass which through excitation has become spherical, or, to speak more generally, has become so reduced as to expose the least possible external surface, all the inotagmata become simultaneously relaxed, after the removal of the excitation, a visible change in the form of the whole mass will not necessarily take place. As a general rule such change will only take place when large groups of inotagmata parallel to one another become partially relaxed only, or still more if they do not relax simultaneously or do so with unequal force. The spherical condition of a naked protoplasmic body can thus correspond as well with complete repose (relaxation) as with maximal excitation (contraction of inotagmata). In addition to the lengthening (relaxation) of definitely arranged inotagma-groups which may very easily lead to the formation of fine pseudopodia, processes may be produced in various other ways. One of the most common is the case described by O. F. Müller in *Amœbæ* and *Amœboid* masses, viz. the very

<sup>1</sup> A word must here be devoted to Kühne's experiments upon so-called artificial muscles ('Unters. ii. d. Protoplasma, &c.,' p. 81), as it claims an importance from a zoophysiological point of view which it would most highly deserve, if the explanation given to it by its discoverer were correct. Before we can concur with this, however, we must have proof, which we have not yet had, 1, that the protoplasmic powder mixed with water and placed in a beetle's intestine develops again to a living excitable protoplasm—a revivification which, according to the best authors, more often fails than not; and 2, that if this first condition be assumed to be fulfilled, all the little lumps of protoplasm fuse into a single organically united mass, for without this the "artificial muscle" is nothing more than an aggregate of independent *Amœbæ* lying against one another, which would not perceptibly change its form on the simultaneous contraction of all these elements. But the experiments showed that the fulfilment of this condition also was an exceedingly improbable event. As, in addition to this, the contents of the "muscle" when emptied out consisted partly of single rounded masses, partly of pale vesicles and free grauaules, out of which no further movements were to be produced, it appears to be fully proved that neither the first nor the second of these conditions was fulfilled.

frequent formation of at first distinctly hyaline spherical prominences into which the granular mass of the interior subsequently streams. Here a general contraction of all the inotagmata of the protruding portion of the hyaline exoplasm may be taken as the cause. As the subsequent unhindered streaming of the granules shows, the cohesion within the hyaline process is very slight and does not sensibly differ from that of a fluid. According to the account first given by Ecker<sup>1</sup> for *Amœba*, which is the one most widely spread among zoophysicologists, the processes are pushed forward by the contraction of the protoplasm lying behind them, especially in the superficial layer. Dujardin supposed and De Bary<sup>2</sup> proved, that the cause of the advance of the mass must necessarily be produced in this case at the periphery of the stream. De Bary found the cause in a there (at the periphery) existing "relaxation or expansion by means of which the granular stream is pulled onwards, either sucked up, just as water is sucked up by a porous body, or simply streaming thither, it being the place of least resistance." Hofmeister also opposed the prevailing view most vigorously, especially upon this ground, that the granular streaming spreads backwards from the periphery where the movement, the extrusion of the process, takes place, i. e. the granules nearest the periphery are the first to move towards it, those farther away commence moving subsequently. It has been correctly remarked that no contraction of those portions of the periphery lying on the other side, the side away from which the body is moving, can be observed, contraction which must necessarily express itself in a smooth stretched superficies which is thus diminished in extent. On the contrary, as may be seen in every amœboid mass which is quickly advancing, the superficial portion of the hinder region of the body, while its volume is constantly diminishing, is not smooth, but wrinkled, folded, if not actually drawn out into fibres.

There is, however, at least according to De Bary,<sup>3</sup> a forma-

<sup>1</sup> Ecker, 'Ztschr. f. w. Zool.,' i, p. 235, 1849.

<sup>2</sup> De Bary, 'Die Mycetozoen,' 2 Aufl., p. 47, et seq., 1864.

<sup>3</sup> De Bary, 'Die Mycetozoen,' 2 Aufl., p. 47, 1864.

tion of processes in a manner corresponding to the older conceptions, in Myxomycetes. But here, according to De Bary, the protoplasm becomes contracted behind the streaming region quite irregularly, the rapidity of the streaming diminishes towards the periphery. In a similar manner local, and especially progressive, contractions of inotagma groups, local differences of pressure, may be produced in the interior of the protoplasm, and following thereupon occur streamings and changes of relative position of those masses which are easily moved.

The theory put forward by Brücke<sup>1</sup> for *Urtica*, that the progressive movement of the protoplasmic contents (granules, nuclei, vacuoles, &c.) always, or at all events generally, takes place in a way analogous to that in which a fluid is moved forward by the contractions of the enclosing pipe walls, is untenable after what we have said.<sup>2</sup>

Note.—It is moreover clear that the formation of processes and streamings may be brought about without physiological contractions, by mere shrinking of the superficial layer (as in partial drying, which for instance occurs not unfrequently in very large plasmodia, or by the coagulation of the albumen, e.g. after ultramaximal electrical excitations). Of course all kinds of combinations of the different means of formation of processes and streamings which have been described may occur especially in experiments with artificial excitations.

### 3. Rotation of the Protoplasm within firm Cell-Walls.

This must take place when the inotagmata of the moving layers are distributed with their long axes parallel to the direction of the movement and a forward movement of the spontaneous stimulus takes place in this direction. The moving

<sup>1</sup> Brücke, 'Sitzsber. d. Wiener Akad. Mathem. Naturw. Cl.,' xlv, 1863.

<sup>2</sup> Cp. also the refutation of the universal applicability of this view by M. Schultze, 'Das Protoplasma, &c.,' p. 51, et seq.; also A. De Bary, "Ueber den Bau u. das Wesen der Zelle," 'Flora,' 1862, p. 249.

protoplasm creeps in this manner over the motionless cortical layer just as a snail's foot over the surface upon which it is crawling.

#### 4. Stoppage of Spontaneous Movements by Artificial Stimulation.

As described above, the first result of artificial stimulation is, as a rule, a standstill or slowing of the already existing spontaneous movements in the spots directly excited. Superficially considered it might appear as if in all these cases no excitation but rather a paralysis had taken place; a view which has been specially urged by vegetable physiologists. The opposite must, however, according to our theory, take place, and it can easily be shown that according to it the result observed and no other would take place. Inasmuch as all not previously contracted inotagmata fall into contraction as a result of the excitation, the impulses to movement at all points of the protoplasmic mass become essentially equal, the entire mass thus immediately assuming a position of equilibrium. It is just the same as if all the muscles of an animal were simultaneously stimulated to the maximum; the external appearance in this case being a standstill, a stoppage of the normal movements.

Hitherto, in referring the movements of protoplasmic masses to active changes of form of the smallest component particles, we have merely postponed the solution of the real difficulty, which consists rather in an explanation of the mechanism of these changes of form. Here we must limit ourselves for the present to a few notes, which must be regarded as hints for further investigations rather than as any solution of the problem.<sup>1</sup>

For reasons already assigned the mechanism concerned can be no other than that mechanism which lies at the basis of active changes of form of muscles and ciliary organs. With regard

<sup>1</sup> Cp. in this respect, *Arch. f. d. ges. Physiol.*, vii, p. 33, et seq., 155, et seq. (especially p. 176), 1873; viii, p. 95, et seq., 1874; xviii, p. 1, et seq., 1878.



to muscles, we can now no longer doubt that changes of form of their contractile particles go hand in hand with changes in the amount of their water-contents, i.e. with the extent to which they are swollen by imbibition. As can be proved, the contractile, doubly-refracting bands of transversely striated muscular fibres swell up during vital shortening by the imbibition of fluid from the isotropic, non-contractile bands lying between them, and on relaxation this fluid is given up again to the latter. Vice versâ, the characteristic shortening can be produced by artificially caused swelling of the doubly-refracting discs of muscles, even when they are no longer excitable. The same is the case with cilia. It is now a generally accepted rule that anisodiametric, doubly-refracting organised animal and vegetable structures, whether living or dead, tend to shorten on the imbibition of water (swelling—*Quellung*)—often with very great force and always in the direction of the optical axis. We may assume that the proximate cause of the change of form of inotagmata of the protoplasm (as of those of other contractile substances) is a change of their water-contents, and thus look upon the cause of contraction as a peculiar process of swelling (*Quellungsvorgang*).

Hofmeister<sup>1</sup> has already, starting from the changeability of the imbibition condition of the protoplasm, looked for the cause of its movement in periodical changes of the water-contents of the smallest protoplasmic particles, and carried this theory out thoroughly in an original manner. He assumed, however, only volume- and not form-changes of the smallest particles, which does not suffice to explain the great amount of the shortening or lengthening observable in many cases. He was not then acquainted with the process of “swelling” which takes place in the contraction of muscles.

In so far as the process of contraction has been hitherto referred to a process observable in undoubtedly lifeless bodies (e.g. connective-tissue fibres dried or hardened in absolute alcohol) the further analysis of the mechanism must be left to

<sup>1</sup> W. Hofmeister, ‘Die Lehre von der Pflanzenzelle,’ p. 63, et seq., 1869.

the physicist. From a physiological point of view the further question would arise, how the changes in the water-contents of inotagmata which are accompanied by the physiological phenomenon of contraction are occasioned. This is probably the point at which the chemist must take up the question. It is, however, in the present state of our knowledge, idle to express any further opinions upon the subject.

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## Contributions to the Anatomy of the Hirudinea.

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With Plates XXIV—XXXIV.

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### i. INTRODUCTION.

IN addition to recording my own observations, which have extended over a period of four years, I have endeavoured to sift and summarise previous observations and to insert them, so as to enable me to give a connected account of the systems with which I have dealt, for the whole group.

The genera with which I deal are :

#### RHYNCHOBDELLIDÆ—

Pontobdella, Leach, 1815.

Piscicola, Blainville, 1818.

Clepsine, Savigny, 1817.<sup>1</sup>

<sup>1</sup> I have retained the name Clepsine because it has such a wide-spread use, but Glossiphonia, Johns., 1816, appears really to have the precedence.

Branchellion, Savigny, 1817.

GNATHOBDELLIDÆ—

Aulostoma, Moquin-Tandon, 1826.

Hæmopis, Savigny, 1817.

Hirudo, Blainv, 1827.

Hæmadipsa, ?

Nephele, Savigny, 1817.

Trocheta, Dutrochet, 1817.

Material.—With the exception of Branchellion, Hæmopis, and Hæmadipsa, all these genera are to be found in England.

Pontobdella is thrown up on the beach or brought in on the skate-fish from time to time upon our South and East Coasts.

Piscicola is to be found upon various species of freshwater fish. I received numerous examples from the Duke of Wellington's trout-breeding establishment at Strathfieldsaye.

Clepsine is very common in ditches and streams in England.

Of Branchellion I obtained four living specimens only during three months spent at the Stazione Zoologica at Naples in the early part of 1882, and in 1883-84.

Pontobdella I obtained in large quantities at Naples, and kept it living in the aquaria there.

Aulostoma is the commonest of our pond leeches.

Of Hæmopis I obtained a single specimen in the neighbourhood of Naples.

Hirudo, although not so much used as formerly, is exported in enormous numbers from Bourdeaux and other places. I have also taken one or two specimens near London, one from a pond near Putney Heath.

Hæmadipsa, the pond-leech of Ceylon and Japan, was brought in a living condition by Dr. C. O. Whitman to Naples, and he very kindly placed some specimens in my hands.

Nephele is abundant in the mud of ponds and streams in England.

Trocheta is a very rare leech in this country. The majority of the authentic specimens have been picked up in Regent's Park, London, possibly imported from abroad in earth or clay,



brought to the Zoological or Botanical Gardens. I have, however, obtained one specimen from a ditch by the side of the Epsom and Leatherhead Railway.

**Literature.**—The literature of the group is enormous in its quantity, and extends back to the middle ages. The importance of leeches in medicine induced numerous observers to work at the group.

I do not propose to deal with any of the older writers, except in one or two special cases, but to take as my basis Moquin-Tandon's classical work '*Monographie de la famille des Hirudinées*,' 1846. Reference is there made to all the older literature.

It is since this date, however, that histological observations of any value have been made, Leydig taking the lead in this respect.

I append a list of the chief works published since 1846, and those to which I shall constantly make reference. The arrangement is chronological.

1. LEYDIG.—"Zum Circulations und Respirations System von Nephelis und Clepsine." '*Berichte von der Königlichen zootomischen Anstalt zu Wurzburg*,' Leipzig, 1849.
2. LEYDIG.—"Zur Anatomie von *Piscicola geometrica* mit theilweiser Vergleichung anderer einheimischer Hirudineen," '*Zeitschrift für Wissenschaftliche Zoologie*,' Band 1, 1849.
3. BUDGE.—"*Clepsine bioculata*, Savigny," '*Verhandlungen des naturhistorischen Vereins der preussischen Rheinlande*,' 1849.
4. GRATIOLET, PIERRE.—"Recherches sur l'organisation du Système Vasculaire de la Sangsue Médicinale et l'Aulastome Vorace" (preliminary note), '*Annales des Sciences Naturelles*'—"Zoologie," xiv, p. 189, 1850.
5. LEYDIG, FRANZ.—"Anatomisches ueber Branchellion und Pontobdella," '*Zeitschrift für w. Zoologie*,' Bd. 4, 1851.
6. QUATREFAGES, A. DE.—"Études sur les types inférieurs de l'embranchement des Annelés. Mémoire sur le Branchellion de D'Orbigny," '*Annales des Sciences Naturelles*'—"Zoologie," 3 Ser., 17, 1852.
7. ÉRRARD, LE DR. E.—"Nouvelle Monographie des Sangsues Médicinales," Paris, 1857.
8. GRATIOLET, PIERRE.—"Recherches sur l'organisation der Système Vasculaire dans la Sangsue Médicinale et l'Aulastome Vorace," '*Annales des Sciences Naturelles*'—"Zoologie," 4 Ser., 17, 1862.

9. LEUCKART, RUDOLF.—‘Die Menschlichen Parasiten,’ Leipzig und Heidelberg, 1863.
10. LEYDIG, DR. FRANZ.—“Vom Bau des thierischen Körpers,” ‘Handbuch der vergleichenden Anatomie,’ Erster Band, Tubingen, 1864.
11. BIDDER.—‘Untersuchungen ueber das Blutgefässsystem einiger Hirudineen,’ Dorpat, 1868.
12. VAILLANT, LÉON.—“Contribution a l’Étude Anatomique du Genre Pontobdelle,” ‘Annales des Sciences Naturelles’—“Zoologie,” 5 Ser., 13, 1870.
13. GEGENBAUR, CARL.—‘Elements of Comparative Anatomy’ (Lankester’s edition), London, 1878.
14. WHITMAN, CHARLES OTIS.—“The Embryology of Clepsine,” ‘Quarterly Journal of Microscopical Science,’ vol. xviii, New Series, 1878.
15. HOFFMANN, DR. C. K.—‘Untersuchungen ueber den Bau und die Entwicklungsgeschichte der Hirudineen,’ Haarlem, 1880.
16. LANKESTER, E. RAY.—“On Intra-epithelial Capillaries in the Integument of the Medicinal Leech,” ‘Quarterly Journal of Microscopical Science,’ vol. xx, New Series, 1880.
17. LANKESTER, E. RAY.—“On the Connective and Vasifactive Tissues of the Medicinal Leech,” ‘Quarterly Journal of Microscopical Science,’ vol. xx, New Series, 1880.
18. BOURNE, A. G.—“On the Structure of the Nephridia of the Medicinal Leech,” ‘Quarterly Journal of Microscopical Science,’ vol. xx, New Series, 1880.
19. LANG, DR. ARNOLD.—Der Bau von Gunda Segmentata und die Verwandtschaft der Plathelminthen mit Cœlenteraten und Hirudineen,” ‘Mittheilungen aus der Zoologischen Station zu Neapel,’ Bd. 3, 1881.
20. SCHULTZE, OSCAR.—“Beiträge zur Anatomie des Excretions Apparates (Schleifenanäle) der Hirudineen,” ‘Archiv für Mikroskopische Anatomie,’ Bd. 22, 1883.

## ii. EXTERNAL CHARACTERS.

The question which I attempt to answer here may be thus stated:—How far in the series of Hirudinean genera do external characters express the metamerically segmented nature of their organisation?

The essential difference between the annulations of a leech and those of a bristle-bearing worm seems to have first struck the mind of De Quatrefages,<sup>1</sup> while Gratiolet<sup>2</sup> for *Hirudo*

<sup>1</sup> ‘Ann. Sci. Nat. Zool.,’ 1852, p. 285.

<sup>2</sup> ‘Ann. Sci. Nat. Zool.,’ 1862, pp. 178, et seq.

and Vaillant<sup>1</sup> for *Pontobdella* have endeavoured to show that the true metamerism of the Leeches is expressed not by the single annuli, but by the recurrence of cutaneous somites consisting of groups of three to five annuli.

My results are completely in accordance with such a view, but I am able to show that there is a uniform law affecting the whole group.

The external evidences of metamerism are most readily studied in *Pontobdella*, and although at first sight specimens appear to differ very much, a closer examination shows a universal uniformity.

The papillæ upon the surface may be very pronounced, conical with a large base and branched apex, or they may be so far drawn in as to leave the animal almost perfectly smooth, or they may be in any condition intermediate between these two extremes.

The complete and remarkable change in the extent to which the papillæ are protruded may be easily observed upon living specimens, and preserved specimens exhibit very pronounced papillæ, or are almost smooth, according as they were killed suddenly or very gradually. The specimen figured (fig. 1.) represents an average condition.

Vaillant (l. c.), who discusses the earlier observations of Savigny, Leach, Baster, and Moquin-Tandon with regard to this point, when speaking of the species he considers to be *P. verrucata*, points out that taking, for example, the somite following the clitellum (ceinture) it consists of four annuli. The most anterior of these bears upon each side of the dorsal median line two large papillæ, while upon the three following annuli the tubercles are less pronounced and so arranged that the two papillæ on either side of the median dorsal line upon the second annulus are placed slightly nearer together than the corresponding papillæ upon the first annulus, those of the third annulus farther apart than those of the second, while those of the fourth annulus are even nearer the median dorsal line than either of the foregoing. I have obtained three, perhaps four,

<sup>1</sup> 'Ann. Sci. Nat. Zool.,' 1870.

species which may be referred to the genus *Pontobdella* at Naples, but only one of these is at all common, the others are, indeed, exceedingly rare, four or five specimens only having come to hand during several years; this small number is, however, probably owing to the difficulties which exist in searching for them in the fish market. I propose now to speak of one species only, *Pontobdella muricata*.

The ordinary somite here presents the following characters:

1. External.—There are four annuli marked by rings of papillæ; other annulations may be present, but careful examination and comparison of numerous specimens show that these are secondary in nature, due to a state of contraction.

The first annulus (fig. 6.) (i) presents eight large papillæ, i. e. papillæ which, when extruded, are large, or when flattened present a large basal marking. These are arranged at regular intervals, except that in the median dorsal and ventral lines there is a more marked gap, so that they may be considered to be arranged in two lateral groups of four on each side.

The second annulus (ii) presents twelve papillæ, smaller than those in the first annulus; of these, four lie in a group in the median dorsal region, and the remaining eight form a continuous series around the lateral and ventral regions of the annulus. Between the dorsal group and the others lies on each side a smaller papilla slightly posterior in position to the series.

The third annulus (iii) is much narrower, and its papillæ much smaller; they are twelve in number and thus grouped:—Two near together in the median dorsal line, a gap, and then one on each side, another gap, and then two more, the remaining two being median and ventral in position.

The fourth annulus (iv) resembles the second in all points, except that the lateral ventral series contains only six papillæ instead of eight, there being altogether ten large and two small instead of twelve large and two small.

Such is the normal somite, and this arrangement obtains with exceeding regularity; here and there a little shifting in position may occur or small extra papillæ be visible, but very rarely.



2. Internal.—The first annulus (fig. 8.) (i) lodges the nerve-ganglion of the somite (*gn.*). The funnel of the nephridium (*neph. fun.*) lies in the posterior portion of the annulus. The testis (*t.*) lies in the region of annulus iii.

The dissepiments figured by Vaillant have not the importance which he attaches to them; they are the groups of dorso-ventral muscles, and occur three or four to every somite rather than as he has figured them.

The anterior sucker (figs. 1 and 2, *a.*) may be regarded as prostomial fused probably with the first annulus of somite 1, following this come the other three annuli of this somite and then, successively, three complete somites (2, 3, and 4), the first and only annulus of somite 5, the clitellum (somites 6 and 7), and then 13 somites (8—20) in front of the anus, making 20 well-marked somites. Somites 19 and 20 are much reduced, presenting only two annuli each. The region lodging the anus represents several somites, which were probably present in an ancestor, but are now completely obliterated.

Thus, the somites at the anterior and posterior portions of the body and the clitellar somites become more or less reduced and fused.

The clitellum (fig. 7.) presents at either extremity a small ring devoid of papillæ (*a, b*), possibly with some special relation to the formation of the cocoon.

The first annulus of the first clitellar somite presents eight papillæ with an arrangement differing but little from that obtaining in annulus i of an ordinary somite.

The second annulus presents six larger papillæ and two smaller. Between these two annuli is lodged the male generative pore (*g. p.*, ♂), which is transversely oval with protruding lips.

The posterior clitellar somite presents an almost exact repetition of this arrangement, the female generative pore (*g. p.*, ♀) being lodged between the two annuli and being rather smaller than the male pore.

An examination of fig. 3 will show that a similar condensation of the internal organisation takes place in this region

The single annulus of somite 5 lodges the fifth post-oral nerve-ganglion (*g n.*, 5); close to the male pore, i.e. in annulus i of somite 6 (first clitellar) lies ganglion 6, while by the female pore in a similar position lies ganglion 7, and in annulus i of somite 8 lies as usual the next ganglion (8). In the posterior region of the body the somites become much crowded together, as also do the nerve-ganglia, and it is no longer possible to correlate them.

Counting the sub-œsophageal ganglion (as Vaillant does not) there are twenty-three post-oral ganglia here as in all other members of the group which I have examined.

Branchellion.—Turning now to the consideration of this genus and examining the somite following the clitellum, we find the first annulus bearing so-called lateral or branchial appendages which possess vascular dilatations ("Mamelon," De Quatrefages), while no vascular dilatation is present in those of the succeeding two annuli; these three annuli compose the somite, the next following annulus bears appendages with vascular dilatations. The existence of a system of vessels in the most anterior annulus of a somite in *Pontobdella*, exactly corresponding to the system of afferent and efferent (cf. *infra*, vascular system), vessels of these dilatations in *Branchellion*, completely justifies the view of the homology of the somite comprising three annuli in *Branchellion* with that comprising four annuli in *Pontobdella*.

*Hirudo*.—The external segmentation is not so well marked, the annuli are not to be distinguished inter se to the extent that they are in *Pontobdella*, but a comparison of Moquin-Tandon's and Ebrard's figures of varieties, allowing in a few cases for inaccuracies which as they had not given special attention to this point, might well have crept in, shows that a very regular distribution of the coloured markings in metameric repetition exist, the somite so indicated consisting of five annuli (fig. 4).

Every fifth annulus presents in all varieties which I have examined a ring of white dots as pointed out by Gratiolet (*l. c.*). This is the first annulus of a somite. This annulus, as I have

determined by series of section through it, the preceding and following annulus, lodges the ganglion. The testis lie partially in this annulus, but by far the greater part of it lies in the second annulus. The nephridial funnel, as I shall show below, lies upon the wall of the testis, and thus has a similar position to that obtaining in *Pontobdella*. The nephridial pore to the exterior lies in the last annulus of a somite, while in *Pontobdella* it opens just within the first annulus of the next succeeding somite (fig. 4).

I have never seen a clitellum in *Hirudo*; but in *Aulostoma*, which exactly corresponds in other respects, it commences in the annulus immediately following upon the third pair of nephridial pores, extends over exactly three somites (5, 6, and 7), and the generative pores lie in the second annulus of the second and third of these somites respectively.

Anteriorly, if we suppose that two annuli are missing (we have seen a similar condition in *Pontobdella*), and have been fused in the formation of the mouth, we have the first somite presenting three annuli: the second is normal with five annuli. The clitellum is thus seen to extend over somites 5, 6, and 7, the male pore to lie in somite 6, and the female in somite 7 (see fig. 4, *g. p.*, ♂, and *g. p.*, ♀). Posteriorly the somites are crowded together (Leuckart's observations upon the fusion of primitively distinct ganglia in this region must be remembered in this connection), but twenty somites may be counted. The anus lies well forward in a region following the nineteenth somite. This region must be regarded as representing in a fused condition at least seven ancestral somites.

Thus, *Pontobdella* and *Hirudo*, which I choose as representing the extreme conditions, Branchellion excepted, with regard to external characters, show such characters to be almost absolutely identical. This seems to me a striking result, the meaning of which I shall refer to later on.

The other *Gnathobdellidæ* which I have examined in this respect present characters almost identical with those of *Hirudo*.

## iii. SKIN—EPIDERMIC GLANDS—SENSORY CELLS—DERMIS.

The previous observations upon the histology of the skin are to be found scattered through the works of Leydig, and a complete account of the epidermis in *Hirudo* in Professor Lankester's memoir (16).

The skin consists of:

1. Cuticle.
2. Epidermis.
3. Sub-epidermis or dermis.

Cuticle (see figs. 9—14, *cu.*).—This presents similar characters throughout the group; it is a structureless, hyaline, somewhat elastic envelope, marked here and there with striations. It is easily separable by maceration. Besides the pores in it, which correspond to the mouth, anus, generative apertures, and the apertures of the nephridia, it exhibits all over its surface small rounded pores which correspond to the apertures of the various unicellular glands described below.

In some genera—*Clepsine*, *Piscicola*, and *Pontobdella*—the skin can be voluntarily thrown into papillæ at certain definite spots. In such condition the cuticle becomes much stretched at these spots, but these can be completely withdrawn, the surface becoming perfectly smooth as in *Hirudo*, *Hæmopsis*, *Aulostoma*, *Hæmadipsa*, *Nephelis*, and *Trocheta*, thus bringing very markedly into play the elasticity of the cuticle.

The cuticle is continually undergoing regeneration, new layers being secreted by the epidermic cells, the old cuticle being peeled off. This process is most easily seen in *Hirudo*, which, when kept in cavity, is continually shedding its skin. This cuticular ecdysis takes place, no doubt, in all leeches, but is more frequent in some genera than in others.

Epidermis (see figs. 9—14, *ep.*).—This consists throughout the group of a single layer of nucleated cells; certain of these may become converted into unicellular glands,



which may either preserve their position in the series or may sink into the deeper layers (cf. *infra*—unicellular glands). Two varieties of connective tissue may intrude upon the series of epidermic cells and actually force their way up to the cuticle—pigmented connective-tissue cells (figs. 9—14, *pig.*) and capillaries (*l. c. cap.*) of the vascular system.

No pigment is ever developed in the epidermic cells themselves, as was, on the contrary, shown to be the case in the epidermic cells of *Peripatus* by the late Professor Balfour.

The epidermic cells are always columnar, but vary much in size and shape.

In the Rhyncobdellidæ they are short and wide, and possess a large nucleus, while in the Gnathobdellidæ they are more specialised and longer cells with small nuclei, and are more closely packed together.

*Piscicola* (fig. 11) presents the most primitive arrangement of the epidermic cells. They are small, and with the exception of those which become glandular, to be described below, are very regular in size. Their inner extremities are rounded off. Instead of being closely packed, a small space exists between neighbouring cells. The nuclei are relatively very large. No connective tissue, either in the shape of pigmented cells or of blood capillaries has penetrated between the cells.

In *Clepsine* (fig. 10) the cells remain fairly regular in size, but are more closely packed than in *Piscicola*. The nuclei are relatively smaller.

The amount of pigment which intrudes upon the series varies in the different species, some of which possess much more colour than others; in *Clepsine bi-oculata* (fig. 10) no intrusive pigmented tissue occurs. There are no intrusive capillaries.

In *Pontobdella* (fig. 9) the cells vary greatly in size, but all retain large nuclei: a considerable amount of intrusive pigmented tissue occurs, and capillaries may be seen forcing their way in between the bases of the cells.

In *Branchellion* (fig. 12) the cells vary immensely in size and shape; in the dorsal region they are similar to those of

*Pontobdella*, but in the ventral region lose their columna character, and are much flattened.

The amount of intrusive pigmented tissue is so great that even in very thin sections the epidermic cells are hardly visible. There are no intrusive capillaries.

In the *Gnathobdellidæ* we find little difference in the epidermic cells among the genera *inter se*. The cells are long and narrow, and the nuclei are very small. There is naturally much variation in the amount of intrusive pigmented tissue, this depending upon the amount of colouring in the species, but differing from point to point in accordance with the pattern upon the surface.

In all the genera capillaries force their way in between the separate cells, the epidermis thus becoming a vascular membrane.<sup>1</sup>

In *Nephelis* and *Trocheta* the epidermic capillaries (fig. 14, *cap.*) are exceedingly large and run for considerable distances immediately underneath the cuticle. Very little rough handling in the living state is sufficient to cause extravasation.

Two kinds of modification may take place in epidermic cells :

1. They may become glandular.
2. They may become sensory.

**Epidermic Glands.**—These are of two kinds : (i) mucous glands which remain dermal in position ; and (ii) glands which secrete some special substance, and which take up a deep position, either between the bundles of muscular fibres or even entirely within the muscles of the body wall. Both varieties always remain unicellular.

i. **Mucous Glands.**—These occur all over the surface of the body. They are easily recognisable as modified epidermic cells. As is usual in all similar cells which store up their secretion within themselves (e. g. fat-cells), the nucleus becomes pushed up against the cell membrane, and is surrounded by a

<sup>1</sup> E. Ray Lankester, 'Quart. Journ. Micr. Sci.,' 1880, p. 303 ; and 'Zool. Anz.,' 1880, No. 49.

very thin layer only of protoplasm, the remainder of the cell being filled with the secreted substance.

In *Piscicola* (fig. 11, *gl. m.*) these mucous glands are very numerous, but they never extend below the level of the other epidermic cells; they lie in the same series, and are only distinguishable by their greater breadth, the position of the nucleus and their contents.

In *Hæmadipsa* the majority remain small and retain their position among the epidermic cells.

In other genera they become so large as to be completely forced out of the epidermic series, and lie in the sub-epidermic layer. They obtain a larger size in the *Rhyncobdellidæ* than in the *Gnathobdellidæ*.

When seen in sections some appear to have almost clear or very finely granular contents, and generally remain almost unstained. This difference may be due to the state of activity, and be comparable to the difference observable in glandular cells of the pancreas or gastric glands at different periods; but I am inclined to think that there is some difference in the nature of the secretion, and that the clearer cells are always smaller and more superficial (figs. 9—14, *gl. m.*).

ii. Glands which have taken up a deep position present at least three well-marked varieties, depending upon the nature of the secretion.

a. Salivary Glands.—These occur in all the genera in the region of the pharynx, whether that be protrusible or not; they occur almost as far back as the generative organs. They are exceedingly numerous and large, with coarsely granular contents; they possess very long ductules which open, in the *Gnathobdellidæ*, on the surface of the ridges which bear the teeth, and, in the *Rhyncobdellidæ*, in the walls of the pharynx. When the pharynx is protruded they are stretched, and when it is withdrawn they are thrown into convolutions.

Figs. 16—24 show salivary ducts (*gl. s. d.*). Also woodcut, p. 437. For salivary glands in *Hirudo* see this Journal, vol. xx, 1880, fig. 13, Pl. XXV.

β. Clitellar Glands.—Behind the “salivary” region, both

within the clitellar region and behind, it there are numerous deep-lying glands — clitellar glands. They are absent in *Clepsine* alone, this leech laying its eggs without any cocoon.<sup>1</sup>

In the *Gnathobdellidæ* they are very numerous in the clitellar region, and are placed in groups of four or five in the connective tissue between the bundles of muscular fibres (figs. 13 and 61, *gl. c.*). Their ducts are radially arranged, and open upon the surface of the clitellum.

In *Piscicola*, *Pontobdella*, and *Branchellion* they are very extensively developed; they occur in masses within the longitudinal muscles of the body wall, and the region of their occurrence extends from the clitellum backwards almost to the posterior end of the body. They attain relatively, and in *Pontobdella* and *Branchellion*, actually huge dimensions (figs. 9 and 63, *gl. c.*).

Their ducts which, as in the case of the mucous glands, are mere prolongations of the cell membrane, are arranged in bundles, and run forwards to open at the clitellum. In *Pontobdella* (figs. 9 and 63, *gl. c. d.*) there are some twelve bundles, each containing as it approaches the clitellum some 200 to 400 ductules. Some of these ductules and gland-cells take up a very deep staining; others do not, the difference depending here, doubtless, upon their state of activity. In teased preparation from the fresh animal it is easy to cause the granular contents to flow along the ductules by pressing upon the coverglass.

λ. Prostomial Glands.—A third variety of these deep-lying unicellular glands exists in *Hirudo*, *Aulostoma*, and *Nepheleis*, doubtless in all the *Gnathobdellidæ*. They are situated in the prostomium and the region around the mouth. They form a very distinct group, and send their ducts forwards to open all round the edge of the buccal cavity. They are perfectly distinct from the salivary glands, there being a considerable space between the most posterior of these and the most anterior of the salivary glands. Their contents, moreover,

<sup>1</sup> C. O. Whitman, 'Quart. Journ. Micr. Sci.,' 1878, p. 224.



remain absolutely unstained by borax-carminé, while the contents of the salivary glands take up a very deep staining.

They do not exist in *Piscicola*, *Pontobdella*, *Clepsine*, or *Branchellion*.

I am unable to determine absolutely their function. Observations (Moquin-Tandon, Carena, &c.) show that the anterior extremity is used in fastening the cocoon; it seems possible that the substance secreted by these glands may be used in this operation. If this be the case, why are they not developed in *Pontobdella*? Observations by M. Donadieu, which are cited by Vaillant, show that the cocoon is seized by the anterior sucker and pressed against some object, the animal remaining in this position for some time. I do not consider it at all likely that any secretion comes from the mouth, that, to quote Vaillant "the pretended salivary glands" have anything to do with this process, still less can any importance be attached to the view of Ebrard that the nephridia (*organes de la mucosité*) play any rôle in the matter.

I think that so far as *Pontobdella* is concerned the entire material for the cocoon must be secreted by the "clitellar" glands, and that the anterior sucker can only function in its moulding and attachment, the animal remaining in the position described while the outer surface of the plastic substance hardens.

While working at the zoological station at Naples I kept several living *Pontobdella* in a glass jar and five or six cocoons were deposited,<sup>1</sup> but I was unfortunately never able to observe the process.

We know little of the eggs of *Branchellion* or *Piscicola*, and the eggs of *Clepsine* are not laid in a cocoon.

If *Pontobdella* can form its elaborate cocoon without the aid of these buccal glands, it seems unlikely that they can exist in the *Gnathobdellidæ* (which form simple cocoons) for

<sup>1</sup> The cocoons never developed, and I have learnt from Professor Kleinburg, of Messina, that he has obtained numerous cocoons laid in captivity which have never developed.

this object. Their function must remain for the present as a matter for further investigation.

2. Sensory Cells.—I do not propose to do more here than draw attention to fig. 15, from *Hirudo*, showing certain of these cells and their connections with nerve-trunks. The preparation from which this is drawn was a section cut with a freezing microtome and stained in gold chloride.

My inability to say much upon this subject is the less to be regretted since Leydig has dealt with these simpler tactile bodies and their derivatives, the eyes, in a most detailed manner.<sup>1</sup> In the same place he has given admirable figures of the epidermic glands, both superficial and deep lying in *Hirudo*.

Subepidermis or Dermis.—Between the epidermic layers and the regular circular muscles of the body wall is a layer which varies in thickness in different genera, but exists, to some extent, in all. This may be compared to the subepidermic layer found in the skin of many animals, usually distinguishable in the adult (where its development has been traced, the mesoblast has been shown to enter largely into its composition) by its vascular character.

It is this layer with the superjacent epidermis which rises into papillæ in *Piscicola*, *Pontobdella*, and *Clepsine*, and which constitutes the annular ridges in a contracted *Hirudo*, *Hæmopsis*, &c. It consists of a matrix of connective jelly<sup>2</sup> in which are to be found the various forms of connective-tissue cell to be described below, numerous and large blood-vessels and short muscular fibres; the latter do not occur in *Clepsine*, *Nephelis*, or *Trocheta*, but are very fully developed in *Pontobdella* (fig. 9, *m. cut.*), and serve to raise and depress the papillæ which occur upon the surface of the body. In all the genera the branched ends of the radial and dorso-ventral muscles (see *infra*, Muscular System, p. 435),

<sup>1</sup> 'Vom Bau des thierischen Körpers,' 1864, and Atlas.

<sup>2</sup> For these and some other terms, with regard to the connective tissue, I am indebted to Professor Lankester's paper "On the Connective and Vasifactive Tissues of the Medicinal Leech," 'Quart. Journ. Micr. Sci.,' 1880.

run into this layer terminating just underneath the epidermic layer (see figs. 9—14, *m. rad.*). The contractile blood-spaces which are developed in the lateral appendages in Branchellion and the rudimentary organs of the same nature which exist in Piscicola, Pontobdella, and Clepsine (see *infra*, Circulatory System), lie in this layer.

All the connective and vasifactive tissue elements exist merely as packing to the mucous glands of the epidermis, and in forms where they are excessively developed (e. g. *Hæmadipsa*, the great development being probably connected with its terrestrial mode of life) there is room for comparatively little connective tissue.

#### iv. MUSCLES—PROBOSCIS.

I do not propose to enter here at any length into the arrangement of the body muscles, the older anatomists, Thomas, Spix, Brandt, Dugés, and Moquin-Tandon, with their marvellously patient dissections, have indeed left little to be added to our knowledge in this respect; while more recently Leydig, Leuckart, Vaillant, and Lang have reviewed and added to this knowledge in respect of individual genera.

**Muscles of the Body Wall.**—As in all vermiform animals depending for their power of locomotion upon the muscles of the body wall alone, these muscles are arranged in circular and longitudinal series. The most external layer (figs. 9—14, 61—64, *m. circ.*) is arranged directly transverse to the long axis of the body. Within this layer there may be a series of fibres which have a diagonal course (l. c. *m. obl.*), and within these lies the main mass of longitudinal fibres (l. c. *m. long.*).

There is considerable variation with regard to the arrangement of these fibres in different genera (see figs. 9—14, 61—64), *Clepsine* presenting the simplest arrangement and *Hirudo* the most complicated; in the latter, connective tissue with embedded longitudinal fibres being inserted between the circular and diagonal layers (fig. 13).

**Dorso-ventral and Radial Muscles.**—These fibres belong to one system, the more centrally placed at the sides of the

alimentary canal have been termed dorsal-ventral fibres, while the lateral series are termed radial (figs. 9—14, 61—64, *m. rad.*). They occur in all the genera. They form bundles of fibres which spread out at either extremity, the out-spreading fibres, which are to a large extent formed as branches of the fibres forming the bundle, run through the circular layers or muscle and only terminate close underneath the epidermis (fig. 9, *m. rad.*). The two central rows of bundles pass between the cæcal dilatations of the œsophageal portion (see *infra*, Al. Canal) of the alimentary tract in those genera where such exist.

Leuckart<sup>1</sup> points out that these dorso-ventral muscles separate the leech-body into longitudinal chambers containing alimentary canal and nerve-cord, generative organs, lateral blood-vessels, and bundles of muscular fibres respectively; they are not, however, arranged as walls running longitudinally but as a series of isolated bundles, and it seems more important to note their metameric repetition in series in correspondence with the metameric segmentation of the other organs. Lang has noted this arrangement.<sup>2</sup> They represent the diaphragmatic septa of the Chætopoda. In the region of the pharynx (fig. 16, *m. rad.*) in the Gnathobdellidæ they acquire a special connection with its wall and help in suction (see Pharyngeal Muscles).

**Muscles in the Wall of the Alimentary Canal.**—These are extensively developed in the region of the pharynx only (figs. 16—24). If we compare the proboscis of the Rhyncobdellidæ, not with the pharynx alone of the Gnathobdellidæ, but with the whole body in that region, we find a striking similarity between the two in the arrangement of the muscular and other elements.

The proboscis of the Rhyncobdellidæ is merely the anterior portion of the body which has taken on a special development, and can be withdrawn into a proboscidian sheath; this latter

<sup>1</sup> 'Die Menschlichen Parasiten,' p. 645.

<sup>2</sup> "Neapel. Zool. Stat. Mitthn," 1882, 'Der Bau von Gunda Segmentata;' also 'Archiv de Biologie,' 1881, p. 558.



is not a permanent organ, but a temporary chamber, necessarily formed when the proboscis is retracted.

The mouth (see adjoining woodcut) *m.* is situated at the extremity of the proboscis, *c.*, with its muscular walls, *d.*, these latter being continuous with the walls of the œsophagus, *œ.* The epidermis of the body is directly continuous with the somewhat modified layer of flattened cells covering the membrane, *a.*, which becomes attached to the proboscis at *f.* (see figs. 21, 22, *ep.*). The salivary glands, *gl. sal.*, send their ducts, *d.*, forward to enter the base of the proboscis, and when this latter is retracted the ducts become more or less coiled.

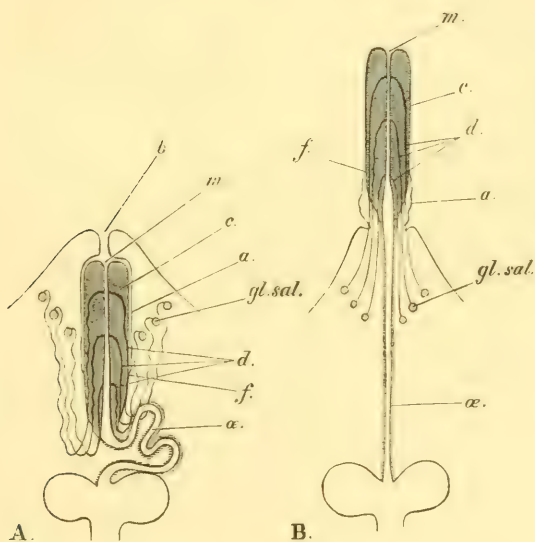


FIG. 1.—Diagram of the proboscis of Clepsine. A. Retracted. B. Protracted. *m.*, mouth; *c.*, wall of proboscis; *b.*, aperture of proboscis chamber; *a.*, wall of the same; *f.*, point where the wall of the chamber is reflected on to the wall of the proboscis; *œ.*, œsophagus; *gl. sal.*, salivary glands (three pairs only represented); *d.*, salivary ducts.

We may now proceed to examine the structure of the wall

of the proboscis, as shown in transverse and longitudinal sections.

Looking, in the first place, at the anterior portion of the body—i. e. in the pharyngeal region—in the Gnathobdellidæ (fig. 16), we find the following structures, and, passing from within outwards, in the following order :

1. Pharyngeal epithelium, *al. ep.* The cells are very minute.
2. Radiating muscles, *m. rad.*, attached to the basement membrane of the epithelium. These pass outwards on all sides through all the other structures, their branched ends abutting upon the epidermic layer.
3. Circular muscles, *m. circ. int.* These form a dense and most compact layer surrounding the three ridges formed by the internal portions of the radiating muscle.
4. Longitudinal muscles, *m. long.*, arranged in bundles separated by connective tissue and by the radial muscles which run through them. Among these occur the sinuses and vessels, salivary glands, and their ducts.
5. Circular and oblique muscles, *m. circ.*, are arranged in an oblique layer and an outer circular layer.
6. Skin, subepidermis, epidermis, *ep.* ; and cuticle, *cu.*

Compare with this the proboscis of the Rhyncobdellidæ (figs. 17—24) :

1. Pharyngeal epithelium, *al. ep.*
2. Radiating muscles, *m. rad.* These are larger than among the Gnathobdellidæ. They pass outwards, and abut upon the epithelium on the surface.
3. Circular muscles, *m. circ.*, developed as in the other group.
4. Longitudinal muscles, *m. long.* The portions of the radiating muscles in this layer are very large ; the bundles of longitudinal muscles are consequently very small, and as the blood-vessels (*cap.*), especially in *Piscicola* and *Branchelion*, are very large in this region, and the ducts of the salivary glands, *gl. s. d.*, numerous, the longitudinal muscles are reduced to a minimum, there being only three or four fibres in the bundle.

5. An external epithelium. This we have seen to be merely the general epidermis of the body, continued into the proboscidal wall.

We have thus very similar arrangements in the two groups, a pharyngeal epithelium and two layers of pharyngeal muscles; and while in the Gnathobdellidæ the layers external to these are well developed, in the specialised proboscis, they have become reduced, the external circular muscles being absent altogether.

The genera of Rhyncobdellidæ present slight but characteristic differences in the structure of the proboscis, which may be seen on comparing figs. 16—24.

In the œsophagus and stomach there are fibres in the wall presenting a not very regular arrangement, but tending to form longitudinal and circular layers. These fibres may be replaced as in *Hirudo* by branched muscular cells. Such cells are described by Leydig<sup>1</sup> as occurring also in *Piscicola*. Somewhat similar cells are also found in the wall of the alimentary canal in Insects and other Arthropods.<sup>2</sup>

Muscles developed in the Walls of Blood-vessels.—Certain of the blood spaces in the Hirudinea present muscular walls;<sup>3</sup> these spaces are such as I shall show below represent the closed vascular system of Chætopoda.

For a description of the arrangement of these fibres in the various vessels see Circulatory System.

Muscles developed in connection with the Generative Organs.—Certain muscular developments found in connection with the penis and vagina, as well as in the walls of the ovisac, are better described in connection with those organs.

Muscles in the walls of the vesicle of the nephridium require no special description.

Muscles developed in the Skin.—Of these I have spoken above.

<sup>1</sup> 'Zeit. f. w. Zool.,' 1849, p. 14.

<sup>2</sup> Leuckart, 'Zootomie,' p. 303.

<sup>3</sup> Leydig, 'Histologie.'

### Histological Characters of Hirudinean Muscle.—

The muscles consist of very long cells arranged either in bundles or lying singly.

These cells may be much branched, e. g. in the wall of the alimentary canal. They consist of a cortical layer which exhibits longitudinal fibrillation (shown in transverse sections), and a medullary substance granular in nature containing a large oval nucleus.

Connective and Vasifactive Tissue.—The comparative study of the histological characters of the connective and vasifactive tissues of the Hirudinea has yielded many interesting results, most of which accord with and elucidate the result obtained by Professor Lankester. I hope to study upon a future occasion the development of the various modifications which exist in the adult, from the simple embryonic tissue. Such a study is necessary to corroborate and amplify the views here put forward which are based upon the phylogenetic relations which exist in the different genera. But even treating the subject in this manner it seems to me possible to trace all the various modifications in their development from the ordinary connective-tissue corpuscle.

Previous observations upon the minute character of these tissues in the group are, in addition to the researches of Professor Lankester upon *Hirudo* above mentioned, to be found in the works of Leydig, who accurately describes and figures pigment cells from *Piscicola* and fat cells from *Piscicola* and *Clepsine*, and of Vaillant, who figures some of the elements from *Pontobdella* but seems to have confounded to some extent the true connective-tissue elements with the glands, epidermic in nature, which lie amongst them. Vaillant's memoir does not in fact profess to be so much histological as anatomical in character.

The Connective Substance in the Hirudinea.—This occurs in all the genera, presenting similar characters but differing in the extent to which it is developed. The amount of this tissue which is developed is in direct proportion to the "limpness" of the leech. *Clepsine* among the *Rhyncobdellidæ* and



Nephelis among the Gnathobdellidæ have only a slight amount of this tissue and are perfectly firm and rigid, almost brittle to the touch while living. Hæmopsis and Aulostoma present the other extreme in this respect, and by no amount of muscular contraction can they render themselves other than "limp," while Hirudo, Trocheta, and Pontobdella present an intermediate condition: usually fairly limp, by muscular contraction they can make themselves rigid. The matrix or ground substance, which, like all other intercellular substance, presents a deep staining after treatment with nitrate of silver, is perfectly hyaline and jelly-like. I have made no very definite observations as to the development of this matrix but there can be but little doubt that it arises by ectoplasmic modification of the corpuscles lying within it. In the youngest Pontobdella I have examined (recently hatched) the corpuscles (fig. 29.) in which are already undergoing various metamorphoses, and are closely packed, there is as yet but little matrix present.

The corpuscles, originally doubtless rounded or but slightly irregular in form, either remain in this condition, undergoing a series of modifications (entoplasmic metamorphoses) while preserving their form; or they may become elongated, and it may be branched, giving rise to fibres, or giving rise to pigment. They may undergo another series of modifications, ectoplasmic and entoplasmic metamorphoses taking place simultaneously, resulting on the one hand in the pigmented patches of the skin, on the other hand in vaso-fibrous and botryoidal tissues. There is probably a fourth line of modification of these primitive cells as the result of which they form capillaries. These are only to be regarded as various lines of modification which may take place simultaneously and to a certain extent overlap one another. They will be best treated separately with reference to the whole group rather than simply described as they occur in individual genera.

1. Entoplasmic metamorphosis—the cell preserving a rounded form. Vacuolated-cells. Fat cells.

The least modified cells are found in Pontobdella and

Clepsine (fig. 25, fig. 9, *a*, and fig. 10, *a*), and are very numerous. They may remain small in size or increase greatly; certain cells of this nature which are relatively enormous occur near the nerve cord. The nucleus always presents a size comparable to that of the cell. The most usual modification which takes place is that a semifluid substance accumulates in droplets in the cell, giving it a reticulate, vacuolated appearance: such cells closely resemble Waldeyer's plasma cells which are found in mammalian areolar tissue (see fig. 9, *b*, fig. 25, *c*).

Another modification is the formation of highly refringent, almost greenish-looking granules, which are arranged in smaller or larger groups (fig. 26). Similar cells occur in *Piscicola*, but in this genus and in *Clepsine* the most common modification results in an accumulation of fat (fig. 27, and fig. 10, *b*). Such cells closely resemble the cells of the developing adipose tissue of *Mammalia*. The fat accumulates in small globules, which run together and form a large globule; the nucleus is pushed to one side, but the protoplasm never disappears so completely as in mammalian fat-cells. In a variety of these cells, which obtain a very large size, the fat-globules never run together, but always remain as scattered globules. The globules present the usual microscopic appearance of fat-globules, and behave in the same manner towards reagents. They stain perfectly black when treated with osmic acid.

These fat-cells do not occur in the *Gnathobdellidæ*. The only genera among the *Gnathobdellidæ* which present these rounded connective-tissue cells are *Trocheta* and *Aulostoma*. They occur in *Trocheta* in enormous numbers (fig. 14, *a*), and, remaining packed closely together, form dense masses. There is, however, a distinct tendency for them to be arranged in rows, probably prior to their conversion into botryoidal tissue (see below).

In *Aulostoma* they are apparently undergoing active metamorphosis, forming simultaneously side by side fibres and pigmented cells (botryoidal tissue) (fig. 28).

2. Ectoplastic metamorphosis. The connective-tissue cells of most widespread occurrence are the elongated or branched corpuscles described and figured by Professor Lankester from *Hirudo* where they are easily studied. They may be even more easily seen in *Hæmopsis*, but best of all in *Pontobdella* (fig. 25, *a*, *b*, and fig. 9, *c*). They occur in all the genera throughout the group.

In young *Clepsine* and *Pontobdella* I have seen them as quite short, stout cells (fig. 29). They doubtless develop from the indifferent corpuscle above mentioned. As they enlarge they become packed with large, highly-refrangent, regularly-arranged globules. These never run together, as do the fat-globules described above. Although they become blackened by osmic acid they are not dissolved by the ordinary fat solvents; they are not, therefore, fat.

The cells present two, three, or four processes, which become immensely elongated and attenuated; so much so that frequently the body of the cell is hardly recognisable. It is these processes and in the *Hirudinea* these alone, which form the fibres which run in all directions in the connective jelly.

In *Pontobdella* I have observed that these fibres may become perfectly elastic in character, appearing twisted and coiled in a teased preparation (fig. 30).

The nuclei of these cells remain small, but I have frequently noticed them undergoing division in *Pontobdella* (fig. 25, *a*).

I cannot pretend to say, in the present state of our knowledge of comparative histology, how far this mode of origin of the fibres from cells in the *Hirudinea* bears upon the mode of origin of the fibres of areolar connective tissue in *Mammalia*. It shows, at any rate, the possibility of their developing by the mere elongation and branching of cells.

3. Ect-entoplastic metamorphosis. The cell develops pigment.

*a.* The cells take no part in the formation of a vascular system. This series of modifications is best seen in *Pontobdella* and the other *Rhyncobdellidæ*. Whether it occurs at

all in the Gnathobdellidæ is a question to which I shall recur.

In *Pontobdella*, outside the walls of the alimentary canal, here and there among the muscles, and in large quantities in the subepidermic tissue, occur cells which preserve to a large extent their rounded form, but which have become closely packed with fine brownish or greenish-brown pigment-granules. These are modifications of the primitive rounded, or perhaps slightly branched, corpuscles. In the adult leech no cells intermediate between the two are to be found, but in the young *Pontobdella* (recently hatched) pigment-granules may be seen forming in such cells. The development of the pigment goes on side by side with the branching growth.

These cells form a complete network in the deeper layers of the subepidermic tissue, being arranged in rows, as are the cells described above in *Trocheta*, and as are also cells which are about to give rise to botryoidal tissue (figs. 35 and 36, and fig. 9, *d*).

Superficial to these in the subepidermic tissue occur cells which are exactly similar to them in their structure, but much smaller and arranged in groups; they appear, in fact, to have arisen by a division of pigment-cells of the lower layer. Their arrangement is very irregular, and they exhibit a tendency to give rise to processes (fig. 37 and fig. 9). Superficial to these, and doubtless derived from them, there is a layer of cells which have become much broken up and branched, some of them becoming perfectly ragged in appearance. Their colouring becomes a trifle darker. It is processes of such cells which force their way in between the epidermic cells, and give rise to the colouring on the surface of the body (fig. 38 and fig. 9, *f*). These three varieties are the only pigmented cells present in *Pontobdella*. They are evidently modifications of the primitive connective-tissue corpuscles which have become more branched and ragged; in other words, degenerate in character as they approach the surface. Similar cells, presenting similar varieties, and similarly disposed, are present in *Clepsine* in varying quantity, according to the species, but never developed to



the same extent as in *Pontobdella*. The rounded cells may attain a huge size in the central regions of the body. The colouring varies, being generally rather greener than in *Pontobdella* (see fig. 10, *c, d*).

In *Piscicola* similar arrangements obtain; fig. 31 represents cells in the deeper subepidermic layers; fig. 32, cells from the more superficial portion. Much branched, cells may also occur in the central region of the body (see Leydig's beautiful figures of cells from the walls of the testes and ovarian sacs.)<sup>1</sup>

In *Branchellion* none of these cells preserve their rounded shape, but all become exceedingly finely branched, and neighbouring cells become joined together by their processes (fig. 34). The fine branches may be seen scattered through the body, but become specially developed in the walls of blood sinuses.

In the subepidermic region and in the branchiæ the development of pigment is carried to the extreme, resulting in the black colouring of, at any rate, the species I have examined. All the pigmented cells present a black pigment in *Branchellion*. It seems to be a general law that the more branched such cells are, the deeper the colour of their pigment.

*b.* The cells take part in the formation of a vascular system, botryoidal tissue, vaso-fibrous tissue. Such an arrangement obtains in all the *Gnathobdellidæ*.

Whether all the cells which develop pigment in the *Gnathobdellidæ* become vascularised, even though they may subsequently lose all connection with the vascular system, is a difficult point to determine. I am inclined to think they do from the manner and region of the body in which they primitively develop.

The mode of development is perhaps best studied in *Aulostoma*, where the processes seem to be actively taking place in the adult animal, doubtless owing to the enormous extent of its digestive powers; a meal which will last a *Hirudo* two

<sup>1</sup> 'Zeit. f. w. Zool.,' 1849, pl. ix, fig. 45, and pl. x, fig. 52; see also fig. 11, *d*.

years being completely digested by an *Aulostoma* in as many days. The process of tissue-growth is more rapid in this than in any other genus.

In the connective tissue in the central region of the body there are numerous rounded corpuscles which appear to be, on the one hand, elongating, forming branched corpuscles, and on the other, to be developing pigment, arranging themselves in rows. A metamorphosis of a portion of their substance forms channels, which afterwards come into communication with other similar channels and with the closed vascular system on the one hand, and with the sinus system on the other, forming "botryoidal tissue." These processes are shown in fig. 28.

The question as to whether the metamorphosis is ectoplastic or endoplastic is one to which I shall revert below.

The structure of the botryoidal tissue has been fully described and figured by Professor Lankester for *Hirudo* in his previously cited memoir, where also will be found an account of the previous observations upon the subject. Its vascular nature was first recognised by Gratiolet in 1862 (*loc. cit.*). Botryoidal tissue presents fairly similar characters in *Aulostoma*, *Hæmopsis*, and *Hirudo*.

In *Nephelis* and *Trocheta* it becomes specially developed (fig. 63). In both genera it is exceedingly abundant and very elastic, being capable of most extensive distension by blood as also it is indeed in the other genera. Its special development consists in its hollowing out to give rise to the remarkable series of vascular dilatations which occur in these two forms—the relations of which I have dealt with below in connection with the vascular system (figs. 63 and 51). These diverticula lodge the nephridial funnels.

*Nephelis* presents the simpler arrangement, the portions of the cells bounding the lumen remaining very irregular, the lumen communicating freely at all points with the lumen of the botryoidal tissue in the neighbourhood.

In *Trocheta* this arrangement is somewhat modified. Actually bounding the lumen is a single layer of pigmented cells regularly arranged. The inner surfaces are slightly irregular

though not so much so as in *Nephelis*, but outside, a regular muscular wall is developed and only one or two definite communications exist with the surrounding botryoidal tissue (fig. 51). As I shall show when discussing the vascular system in *Hirudo*, the blood after entering the more deeply lying portions of the "cutaneous" network (botryoidal tissue) passes into the intermediate portion which consists of vessels with brown pigmented walls; these are constituents of the "vaso-fibrous" tissue (Lankester).

Branches of the botryoidal tissue can be traced passing outwards between the muscles, the cells becoming smaller and the walls very irregular; these are connections between the botryoidal tissue and the vaso-fibrous tissue (figs. 13, 14).

The vaso-fibrous tissue in its turn communicates with the most superficial "cutaneous" network, which is composed of thin-walled capillaries.

The vaso-fibrous tissue is actually developed doubtless from botryoidal tissue, the gradual transition is shown in such connections as the one figured (fig. 13, *k*). The homologous tissues described in *Pontobdella*, which have not become vascularised, are connected in the same way, the deeper lying cells giving rise to the more branched superficial cells.

I would suggest, interpreting my own and Professor Lankester's observations (*loc. cit.*), that capillaries of the botryoidal tissue become converted into capillaries of the "vaso-fibrous" tissue, the cells dividing and remaining small, the walls at the same time thinning and partially breaking down, becoming very irregular, their nuclei dropping into the blood-stream. It seems that this process might be carried further in one of two ways, either—*a*, the lumen might become obliterated (which would account for fig. 4, Pl. XXVII, of Professor Lankester's memoir), the cells or portions of cells remaining would in this case become very finely branched, and, as in *Pontobdella*, distributed in the subepidermic region, penetrate between epidermic cells, and varying in quantity from spot to spot, form the coloured pattern upon the body in *Hirudo*, or—*b*, they might remain as capillaries, the degeneration of the wall being

continued to the extreme, giving rise, in fact, to the thin-walled capillaries. As we find that the regular distribution of pigment obtaining in *Pontobdella* (i. e. the more branched the cell the more superficial its position) disappears to a varying extent in other allied genera, so here, while a regular distribution of pigments holds in *Aulostoma*, this distribution is partially modified in *Hirudo*, branching pigment-cells occurring in great numbers in the walls of the dorsal and ventral sinus (fig. 62), and to a certain extent in other parts of the body; and finally, in *Nephelis* we have an extreme condition (this may well be placed side by side with the extreme condition of *Nephelis* in relation to the development of botryoidal tissue). The branched pigmented cells in that genus become excessively branched, degenerate in character, and as in the similar cases of *Piscicola* and *Branchellion*, dark or even black in colour, and distributed throughout the body.

This theory which accords so well with the blood supply of the parts and with the homology of these cells with those in other leeches, seems to be rendered not only possible but highly probable in the light of the facts which I have brought forward.

4. Entoplasmic metamorphosis. Vacuolation to form capillaries.

The indirect method of capillary formation described above can hardly be universal.

There are certain thin-walled, non-contractile capillaries in direct connection with true vessels (see p. 468), e. g. the capillaries upon the gastro-ileal portion of the alimentary canal which have no relation at all to botryoidal or any pigmented tissue (fig. 47). Until embryological evidence can be obtained respecting this matter, I can only suggest that they are probably formed by the vacuolation of primitive connective-tissue corpuseles. In this connection must be considered the fact that, those leeches where pigmented tissue never becomes vascular, i. e. in forms where no canalisation of pigmented cells has taken place, the blood is always colourless, while in all forms with red blood such canaliculisation of pigmented tissues has



occurred in the formation of the vascular system. I have studied the thin-walled capillaries in *Pontobdella* only. The walls are thin, with apparently a sort of cuticularisation upon the outer surface, while upon the inner surface there appears to be a thin layer of protoplasm, with here and there a nucleus embedded within it (see fig. 47).

The connective tissue of *Branchellion* needs further examination.

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I have now dealt with all the forms of connective and vasive tissue which occur in the adult members of the group which I have examined, and shown that they may all be derived from an indifferent connective-tissue corpuscle—such corpuscle undergoing ecto- or entoplasmic, or even ect-entoplasmic metamorphosis.

We have in the process of the formation of botryoidal tissue an instance of cell-metamorphosis which in all its phases is neither distinctively ectoplasmic nor entoplasmic. It may in certain cases be solely entoplasmic, the vacuolation taking place within the cell, the lumen formed coming into connection with the lumen formed in the neighbouring cells, and so giving rise to the vascular channel. On the other hand, cells lying next one another in two rows may undergo metamorphosis at the edges where the rows join, and the vessel, may thus form in an ectoplasmic manner; but it is obvious that in the vast majority of cases neither one of these processes alone occurs, but a mixture of the two. This is, in fact, a case where it is not so important to distinguish between the two methods of cell metamorphosis as to confine our attention to the lumen itself. Is this lumen intracellular or is it intercellular? We may further consider the general question—is there any well-founded distinction to be drawn between spaces in the animal body with regard to their relations to the cell or cells surrounding them? It is a subject which requires further investigation—a more exact knowledge of histogenesis

than we at present possess, and the following remarks must be regarded as somewhat speculative.

All the cells composing the animal body, however complex, proceed from the fertilised ovum, and are modified in various ways from the primitive embryonic cells. It seems to me, however, that we cannot say so much about the spaces which exist in the animal body.

Some of these, e. g. the contractile vacuoles of Protozoa, the ducts in the nephridial cells of leeches, the newly-developed vertebrate capillary, and all such intracellular spaces, are obviously formed by actual metamorphosis of the cells themselves, and are surely to be contrasted with such spaces as the lumina of invaginated gastrulæ, in the formation of which no cell metamorphosis takes place and which are in fact formed outside cells. The former variety of space may be called endocytic the latter paracytic, and we may distinguish between endocytic cœlosis and paracytic cœlosis. A comparatively small number only of existing histological observations are exact enough to enable us to test the validity of this distinction. To take an instance, it seems to me very probable that there exists an antithesis in this respect between cœlomic and vascular space in many instances, if not as a general rule. In a considerable number of cases true blood-vessels have been stated to develop by a nipping off of cœlomic space, and Bütschli<sup>1</sup> and others consider this to be the rule; but there is an ever-increasing number of observations of vessels being formed either by vacuolation of single cells or by the breaking down of the central cells of a solid cord, the remnants of the cells floating in the newly-formed lumen becoming in many cases blood-corpuscles. This is endocytic cœlosis. The cœlom, on the other hand, is either formed directly by budding from a cavity derived from the archenteron, or as a space between cells, there being no appearance to suggest that any change in the cells themselves—any cell metamorphosis—takes place. Both these processes of lumen formation (cœlosis) may be either :—

<sup>1</sup> Bütschli, 'Morph. Jahrb.,' 1883.

a. Direct, the lumen appearing at once.

β. Indirect, the appearance of the lumen being delayed ; the latter to be considered as a "perturbation" of the former process.

"Direct paracytic cœlosis" occurs in the embolic formation of the typical invaginate gastrula or the auditory involution of Vertebrata. "Indirect paracytic cœlosis" occurs where a solid mass of cells is pushed in and the space then accumulates between them, as in the formation of the archenteron where there has been an epibolic invagination, and in the formation of all segmentation cavities.

"Direct endocytic cœlosis" takes place wherever vacuolation occurs within a single cell as in the developing blood capillary of the Vertebrata, while the process may be termed "indirect endocytic cœlosis" in a case where it is only after that cell has divided and given rise to a group of cells that endoplastic vacuolation or ectoplastic metamorphosis of each individual cell causes the formation of a lumen common to that group. Such takes place in the formation of the botryoidal tissue discussed above, and if I surmise correctly in all instances where a solid cord of cells is formed and the central ones subsequently break down forming the lumen (large blood-vessels of Vertebrata, ? all large blood-vessels, ? the lumen of the heart<sup>1</sup>).

Thus, in all endocytic cœlosis the lumen has necessarily an intracellular origin. Where whole groups of cells, as in the central portion of a solid rod, break down at once, the lumen may appear to be intercellular in origin though really formed by the metamorphosis of cell-substance. On the other hand, in cases where the origin was obviously an intracellular one, the cell in which the lumen was developed may divide and the lumen may be ultimately surrounded by a group of cells, and so may come to be intercellular and indistinguishable in the adult from a paracytic space. Such is the case with the capillaries of Vertebrata: the lumina of the cells run together, the cells divide, and the capillary with its endothelial lining is

<sup>1</sup> See Balfour's account of the formation of the heart in spiders, 'Comp. Anat.,' vol. i, p. 374.

formed. Usually not more than two or three cells, however, surround the lumen in any particular region.

This important question leads us so far away from our subject and rests at present upon such a speculative basis that it is not advisable to discuss it further here.

#### V. BLOOD—BLOOD-CORPUSCLES.

There is only one vascular fluid in the Hirudinea which corresponds to both cœlomic and red vascular fluids as found in the Chætopoda.

Curiously erroneous statements exist with regard to this fluid ; the presence of corpuscles has been universally denied, and a colouring matter has been described where none such exists, and its existence denied in genera where it is always present.

Rhyncobdellidæ.—The blood is colourless. Colourless amœboid corpuscles occur in very large numbers. A nucleus is present surrounded by a considerable amount of granular amœboid protoplasm (fig. 39). This protoplasm often becomes drawn out into filamentous processes, which, as is the case with the majority of amœboid corpuscles, tend while upon the microscope-slide to fuse together.

These corpuscles are probably formed from the walls of the capillary vessels: these in Pontobdella, at any rate, where I have examined them closely (fig. 47), present naked protoplasm upon their inner surface with embedded nuclei. The naked protoplasm presents amœboid processes. These corpuscles are possibly also formed in the "lymphoid" nodules described below (fig. 44). In addition to these amœboid corpuscles there may be constantly seen in Clepsine, floating in the larger blood spaces, much larger rounded cells; these, as I shall show below, are cœlomic epithelium cells which become detached from the walls of the sinuses, while their size prevents them from being carried into the true blood-vessels. Similar cells may be seen in a few places in Pontobdella (figs. 10, *cœl. ep.*; 53, *cœl. ep.*).

Gnathobdellidæ.—The blood is red, the plasma con-



taining dissolved hæmoglobin. In preserved *Nephelis*, hæmoglobin crystals are sometimes found in the vessels. Colourless amœboid corpuscles occur in great numbers. Fig. 40 A, shows these corpuscles from *Nephelis*; fig. 40 B, from *Hirudo* as they appear in the fresh state; fig. 40 C, after treatment with acetic acid, and fig. 40 D, after treatment with magenta. These figures can leave no doubt about their being nucleated amœboid corpuscles.

The blood in all these genera coagulates rapidly when withdrawn from the body—filaments of fibrin or some allied substance may be seen forming upon the microscope-slide.

#### vi. VASCULAR SYSTEM—CÆLOMIC SPACES.

The observations of the older anatomists upon the circulatory system of leeches, observations by Du Rondeau, Knolz, Dutrochet, Thomas, Della Chiaje, Cuvier, Dillon, Spix, J. Müller, Wagner, Dugés, and others, dating from the year 1782, admirably summed up by Moquin Tandon,<sup>1</sup> resulted in a general knowledge of the anatomical relations of four longitudinal trunks and their main branches. No question as to the possible relation of these to "cœlom," i. e. to the perivisceral spaces of other worms, seems to have entered their minds. It was reserved for Leydig<sup>2</sup> to show that in *Clepsine*, *Piscicola*, *Pontobdella*, and *Branchellion*, the vascular trunks form two distinct systems, a contractile and a non-contractile, connected with one another at one or two points only, and for De Quatrefages<sup>3</sup> to suggest the relation existing between these systems and the closed vascular system on the one hand, and the general perivisceral cavity on the other, as they exist in chætopod worms. Leuckart<sup>4</sup> was the first to insist upon the homology between the blood spaces of the group generally and the "cœlom," which had by that time been recognised as of such morphological importance in all the higher animals.

<sup>1</sup> 'Monogr. de la famille der Hirudinées,' 1846, p. 143.

<sup>2</sup> Leydig, 'Berichten k. zoot. Anstalt zu Würzburg,' 1849.

<sup>3</sup> De Quatrefages, 'Ann. sci. nat. Zool.,' 1852.

<sup>4</sup> 'Die Mensch. Parasiten,' 1863.

Valuable as the theories above mentioned were, and minute and detailed as are the descriptions of the vascular system by Budge<sup>1</sup> in *Clepsine*, by Gratiolet<sup>2</sup> in *Hirudo*, by Bidder, in *Nephelis*<sup>3</sup>, and by Vaillant<sup>4</sup> in *Pontobdella*, it is only by the use of the most recent histological methods, and in the light of recent theories concerning cœlomic spaces and blood sinuses, that I have been able to form a consistent scheme of the vascular spaces in the group as a whole (see p. 474).

Leydig's division of the vascular spaces into a contractile and a non-contractile system, appears to hold good, not only, for the four genera specially investigated by him, but, for the whole group, and as De Quatrefages pointed out, the former seems to represent the closed vascular system of *Chætopoda*, while the latter is probably cœlom, whether in the state of new formation, or, gradually becoming occluded, i. e. in a state of degeneration. My object has been to determine throughout the group, what spaces are to be regarded as belonging to one system and what to the other. The whole system being in absolute continuity, it becomes work of considerable difficulty to determine whether any given space or system of spaces belongs to one system or to the other. The presence or absence of rhythmic contractility only possesses importance for the solution of this question in the case of the larger trunks.

The existence of various organs, alimentary canal, nerve-cord, testis, &c., in certain spaces, points quite conclusively to the cœlomic nature of such spaces. The funnels of the nephridia play a very important rôle in this respect, occurring in different spaces in different genera. By the word "cœlom" I understand a space or set of spaces excavated in the mesoblast and distinct from blood-vessels, such as is the body-cavity of *Chætopods* and of *Vertebrates*, and I do not undertake in any way to discuss whether such space is a pseudocœl or an enterocœl

<sup>1</sup> Verhand. Nat. Hist. Vereins. der Preuss. Rheinlande, 1849.

<sup>2</sup> 'Ann. sci. nat. Zool.,' 1862.

<sup>3</sup> 'Untersuchungen über dass Blüttgefässsystem einiger Hirudineen,' Dorpat, 1868.

<sup>4</sup> Ann. sci. nat. Zool.,' 1870.

in the Hertwigs' sense, or may be something altogether unprovided for in the artificial and valueless system of those authors.

#### RHYNCHOBDELLIDÆ: *Pontobdella*.

We owe to Vaillant the most complete description of the vascular system in this genus, Leydig having only made a few isolated observations upon it.<sup>1</sup>

Vaillant's observations, careful as they appear to be, are based chiefly upon separate injections, which have resulted in a complete mixture of the two systems. Vaillant describes (as vessels) the dorsal sinus, the ventral sinus, and the lateral vessels, but does not appear to have seen the dorsal vessels, ventral vessels, nor the traces of lateral sinuses which exist.

**Dorsal and Ventral Sinuses.**—In the anterior region there is in *Pontobdella* a large sinus which surrounds the pharynx and its sac, the brain, and nerve-cords, and in the dorsal and ventral walls of which lie respectively the dorsal and ventral vessels. Between the seventh and eighth post-oral ganglia this becomes narrowed into the ventral sinus. Anterior to this it gives off branches on either side, which, uniting dorsally, form the dorsal sinus (fig. 63, *d. s.*, *d. v.*, *v. s.*, *v. v.* See also woodcut, fig. 2).

The dorsal sinus encloses, for the greater part of its length, the dorsal vessel. I say along the greater part of its length, because here and there the tissue which is filling up the system of sinuses has grown around the dorsal vessel, so that the latter comes to lie side by side with the dorsal sinus. The same remark applies to the ventral vessel and sinus. This arrangement is carried to a greater length in *Branchellion*, and has given rise to statements as to a double character of the dorsal and ventral sinuses.

The dorsal sinus tends to lose its character as a definite sinus in the posterior region of the body, being split up into a network of small sinuses.

In the regions of the ganglia branches of the ventral sinus

<sup>1</sup> 'Zeit. f. w. Zool.,' 1851, p. 319.

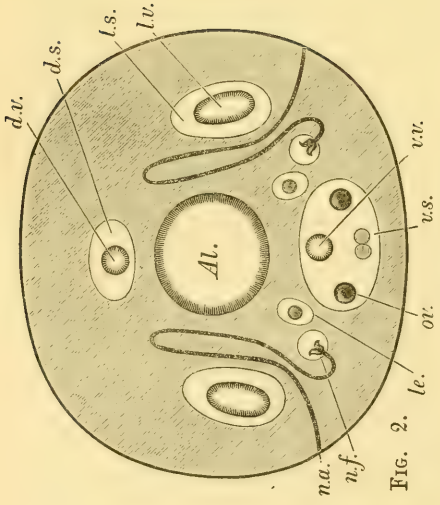


FIG. 2.

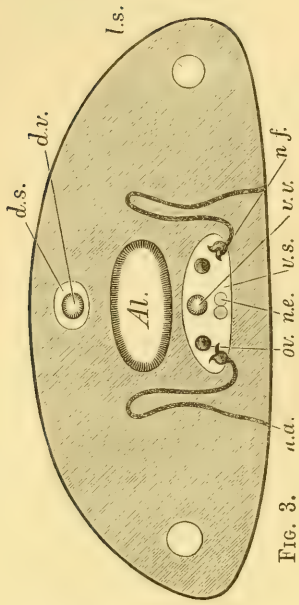


FIG. 3.

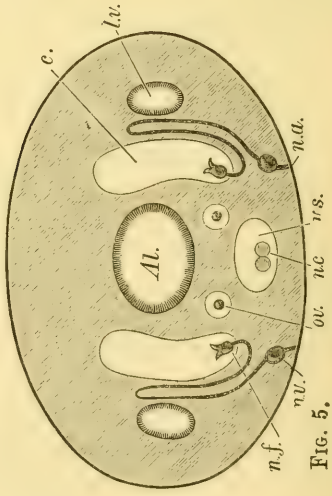


FIG. 5.

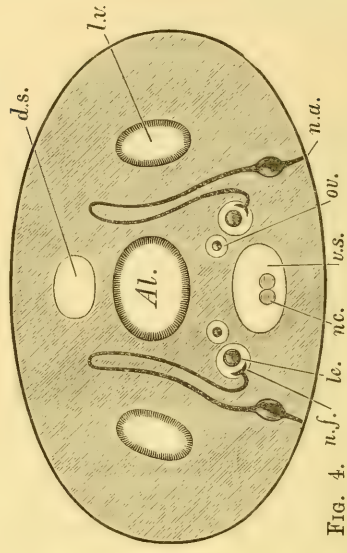


FIG. 4.



FIG. 2.—Diagram of a transverse section of *Pontobdella*.

FIG. 3.—Similar diagram of *Clepsine*.

FIG. 4.—Similar diagram of *Hirudo*.

FIG. 5.—Similar diagram of *Nephelis*.

*Al.* Alimentary canal. *te.* Testis. *ov.* Ovary. *n. c.* Nerve-cords.  
*n. f.* Nephridial funnel. *n. a.* External aperture of nephridium. *d. s.*  
 Dorsal sinus. *v. s.* Ventral sinus. *l. s.* Lateral sinus. *d. v.* Dorsal  
 vessel. *v. v.* Ventral vessel. *l. v.* Lateral vessel.

These diagrams indicate the amount of vascular space which occurs in these various genera, whether vascular or cœlomic. The vascular spaces are marked by a thickened striated contour, while the cœlomic spaces are drawn with the single line limiting them.

arise; upon each side these surround, at their origin, the nerve branches which arise from the ganglia. They then pass in a dorsal direction, and, giving off branches, run towards the dorsal sinus and open into it; anterior branches of the same trunks may do likewise (fig. 8, *d. v. s.*).

In the middle region of the body these dorso-ventral sinuses form spherical dilations a little beyond the point at which the nerve branch leaves the sinus (fig. 8 and woodcut fig. 2, *n. f.*); it is in these dilations that the nephridial funnels are lodged. Beyond this point, in the testicular region, the sinus divides into two main branches. One runs at once towards the dorsal sinus, the other, passing in an anterior direction, forms a dilatation, which almost entirely surrounds the testis (fig. 63, *p. t. s.*, woodcut, fig. 2), a circumtesticular sinus; it then passes to the dorsal vessel.

The ventral sinus encloses, in addition to the organs mentioned above, the ovaries and the greater part of the copulatory apparatus (see woodcut, fig. 2, *v. s.*).

Here and there the dorsal sinus has developed in its wall a muscular band, consisting of some four or five circularly arranged fibres (fig. 45, *m.*).

**Dorsal Vessel.**—The dorsal vessel, as Leydig pointed out, possesses a series of valves similar to those in Clepsine. I have not seen any branches arising from it. At either end it communicates with the ventral and, I think, lateral vessels. I am not quite certain about its communication with the lateral vessels anteriorly. The blood always passes from behind forwards.

**Ventral Vessel.**—This does not give rise to any branches, but communicates anteriorly and posteriorly, as above described, with the dorsal vessel.

**Lateral Longitudinal Vessels.**—Communicating certainly at the posterior extremity, with the dorsal and ventral vessels, these run forwards without any branches to the body; anteriorly they anastomose with one another.

Vaillant describes a connection between the lateral vessels and his dorsal vessel in the somite with the first pair of testes; this he demonstrated by injection. I cannot discuss the mean-

ing of this communication, as I am uncertain whether in this instance Vaillant had hold of the lateral vessel or lateral sinus, or indeed of the dorsal vessel or dorsal sinus. The inference I draw from his description is, that there is a communication from the lateral vessel to the dorsal sinus. This seems improbable, since no other leech presents any such arrangement. If, however, it passes from the lateral vessel into the dorsal vessel, it is no anomaly, and with due reserve I may suggest these to be its relations.

Around the lateral (longitudinal) vessel lies what I have called above a lateral sinus; this is never really developed as a sinus around the lateral vessel, but there is a looseness about the connective tissue outside the muscular wall of the vessel, and a considerable amount of blood in the region, and—a fact which seems to me to possess great weight—there exist around the lateral vessels muscular bands, three to every somite, which recall the muscular bands described above as occurring in the wall of the dorsal sinus (fig. 46, *m.*). I consider that we have here the remnants of a lateral sinus which surrounded the lateral vessel, as the dorsal sinus surrounds the dorsal vessel (woodcut fig. 2, *l. v.* and *l. s.*).

As stated above, no branches pass from the lateral vessel to the body; there are, however, a series of small branches (branchial vessels) which, passing through the muscular layers, open into dilatations lying in the subepidermic region; these dilatations communicate, on the other hand, by a sinus (branchial sinus) with the "lateral sinus"; the degenerate condition of the latter is quite in accordance with the condition of these lateral dilatations. Blood passes from the lateral vessel into these vesicles, and then into the sinus (figs. 8 and 64, *br. v.*, *br. s.*).

In *Pontobdella* certain other very interesting vascular developments occur, which require further investigation. They are rounded bodies, occurring along with the deep-lying clitellar gland-cells, and of about the same size. They present a more or less definite capsule (fig. 44, *o*), formed by a close packing of elongated connective-tissue cells. In certain spots there is free communication with blood spaces (fig. 4, *m. n.*). The capsule,

such as it is, consists of nothing but these cells; there is no endothelial lining occupying the interior of this capsule; there are an immense number of branched connective-tissue cells, whose branches unite, forming a complete reticulum (*loc. cit.*, *p.*) reminding one most strongly of the medullary substance of the lymphatic gland of a Vertebrate; it is possibly a primitive lymphoid tissue.

Between the meshes of this network are packed numerous white blood-corpuscles (*loc. cit.*, *q.*). (The majority of these have been omitted from the figure to show the reticulum.)

We have here morphologically the simplest possible lymphatic glands (more strictly comparable to the lymphatic nodules which occur within or around lymphatics, or in other parts of the body, these nodules lying in what may be termed the lymphatic space); and I think it highly probable that these organs play also the physiological rôle of the lymphatic gland producing the white blood-corpuscles.

The exact mode of origin and relations of these might throw light upon lymphatic glands of Vertebrata. These structures in *Pontobdella* seem to be only special results of that process of diacœlosis which is going on throughout the group—ingrowth of connective tissue “scattering” or carrying with it cœlomic space.

### Clepsine.

The system of blood spaces in Clepsine has formed the subject of a large number of special memoirs by Filippi, O. F. Müller, Grube, Leydig, Budge, Leuckart, Bidder and Whitman.

The most recent author, Whitman,<sup>1</sup> while citing a large number of the older authors, curiously enough has not seen the most complete description, a description in many respects more complete than his own, that by Budge.<sup>2</sup>

I propose to deal with the descriptions by Leydig, Budge, and Whitman.

<sup>1</sup> ‘Quart. Journ. Micr. Sci.,’ 1878, p. 286.

<sup>2</sup> Verh. Nat. Vereins. der Preuss. Rheinlande und Westphalens, 1849.



Whitman, following in the steps of Leydig, separates (1) a closed vascular system from (2) a lacunar system (sinuses, A. G. B.)

**Ventral Sinus.**—This runs the whole length of the body, and contains the nerve-cords, ventral vessel, ovaries, and nephridial funnels (woodcut, fig. 3, *v.s.*; fig. 64, *v.s.*).

**Dorsal Sinus.**—This runs along the whole length of the body as a special channel, communicating at either extremity with the ventral and the lateral sinuses (woodcut, fig. 3, *d.s.*, and fig. 64, *d.s.*).

The great median sinus described by Leydig, containing the dorsal and ventral vessels and alimentary canal, does not exist in Clepsine, nor in any leech throughout the length of the body, but the network of sinuses in this region presents so much blood space and so little parenchymatous tissue that it cannot be a matter of surprise that, basing his observations upon dissections and views of the whole animal as a transparent object, even such an astute observer as Leydig has been misled (see fig. 10, *coel*, fig. 64, *coel*).

**Lateral Sinuses** (marginal sinus, Whitman).—These run longitudinally along the whole length of the body and communicate very freely with one another and with the dorsal and ventral sinuses at the posterior and anterior extremities (fig. 64, and woodcut, fig. 3, *l.s.*)

I have been unable to determine the exact arrangement of the sinuses which connect these main trunks, owing to their very extensive nature—owing, in other words, to the slight extent to which the *cœlom* has become split up or has undergone diacœlosis.

**Dorsal Vessel.**—This lies in the dorsal sinus (woodcut, fig. 8, and fig. 64 *d. v.*). Its structure and the arrangement of its branches have been most minutely described by Budge. It presents in the median region a series of fifteen dilatations with specially developed muscular walls, each provided at its posterior extremity with a valve which was correctly described by Leydig as a group of cells. At its anterior extremity the dorsal vessel bifurcates, the branches pass forwards and toward the ventral

surface and open into the anterior extremity of the ventral vessel.

In the region of the pharynx the dorsal vessel gives off a small trunk which branches upon the walls of this organ in a manner accurately described by Budge. The branches collect again into a vessel which opens into the ventral vessel.

The 2nd, 3rd, and 4th of the dilatations above mentioned give off branches, the anterior of which run forward directly and open into the anterior extremity of the ventral vessel, the posterior run backwards for a considerable distance, as far as the thirteenth post-oral ganglion and then run forwards to open on each side into the ventral vessel close to its anterior extremity.

At its posterior extremity the dorsal vessel gives a series of vessels on each side (four, according to Budge, six, according to Whitman), which looping round on to the ventral surface give rise to the ventral vessel.

Ventral Vessel.—This lies with the nerve-cords, ovaries, and nephridial funnels in the ventral sinus (woodcut, fig. 3, and fig. 64, *v.v*). It is formed anteriorly by the branches of the dorsal vessel above mentioned, receives the efferent pharyngeal vessel and posteriorly, anastomoses with the dorsal vessel as shown above. I may point out in passing, how closely this system of vessels resembles that of *Branchiobdella*. In addition to the vessels and sinuses described above there are certain other blood spaces, the exact relations of which I have been unable to make out:—

1. Budge describes other branches of the dorsal vessel (nine pairs) which supply the walls of the gastro-ileal portion of the alimentary canal, and after anastomosing with one another open at three spots on each side into the lateral sinus. I cannot speak positively regarding these branches, of which Whitman makes no mention. My sections show that such branches exist, but I cannot trace their distribution, and I have not had an opportunity of examining the transparent species, *C. bioculata*, used by Budge; that they supply the intestinal walls is probable

enough, but that they open directly into the lateral sinuses I very much doubt.

2. Leydig figures only eleven pairs of sinuses communicating between the ventral and the lateral sinus, Whitman about twenty, and Budge some sixty-two pairs of such sinuses, only the last-mentioned observer not recognising the median ventral sinus figures these as running from one lateral sinus to the other. My own observations are not clear upon this point. While injecting *Clepsine* with mercury from the ventral sinus I have observed the mercury pass forwards and instantly fill the dorsal sinus and afterwards the lateral sinuses, the mercury has then appeared in about 120 very superficial sinuses which run directly transversely; these appear not to be filled directly from the lateral sinuses and to fill in alternate directions, one filling from right to left, the next from left to right, and so on. I am uncertain at present as to the nature of these, but their number and absolute regularity is very striking. It seems to me probable that Budge saw these spaces, but that he counted a pair as one, while Leydig and Whitman saw the more deeply-lying sinuses passing from the lateral to the median ventral sinus.

3. The above description, unless Budge be correct about the vessels coming from the intestinal walls opening directly into the lateral sinus, provides for no communication between the vascular system and the system of sinuses. That such a communication exists in all leeches appears to me certain from the fact that the blood presents similar characters in vessels and sinuses. Although the large cœlomic epithelium cells which line the larger sinuses in *Clepsine* and *Pontobdella* sometimes float into the blood, they never occur in the vessels—the communications between the latter and the sinuses are no doubt too small to allow of the passage of such large cells. The common corpuscles are of exactly the same size whether they occur in the one system or the other, and although no communication is obvious in *Clepsine*, there is a strong *a priori* probability in favour of its existence, in that, the two systems are so obviously connected in the majority of Hirudinean genera.

There are, moreover, at the lateral margins of the body a series

(?number, about 10) of small dilatations which exhibit rhythmical contractions, and which are homologous with similar organs in *Piscicola* and *Pontobdella*, and I have little doubt with the dilatations at the base of the branchiæ in *Branchellion*. As I have shown in *Pontobdella*, these dilatations form a medium of communication between the two blood-systems, thus rendering it highly probably that there is a similar communication in the case of *Clepsine*.

### *Piscicola* and *Branchellion*.

The slight extent to which I have been able to carry my observations in *Piscicola* and *Branchellion* forbids my criticising the existing observations of Leydig and De Quatrefages, or giving any full account of the circulation; I may, however, record a few isolated observations which seem to connect these genera with *Clepsine* and *Pontobdella* in this respect.

*Piscicola*.—As Leydig has pointed out the dorsal vessel lies in a dorsal sinus.

In its anterior portion it gives off several branches which form the ventral vessel as in *Clepsine*.

I regard it as probable that Leydig is mistaken here, as in the case of *Clepsine*, with regard to the dorsal vessel being open posteriorly; and that, as in that genus, it runs on and communicates posteriorly also with the ventral vessel.

Branches similar to those described above in *Pontobdella* leave the lateral vessel and pass into the skin opening into a contractile dilatation, returning thence to communicate with the sinous system; but this latter is mere conjecture.

### *Branchellion*.

Moquin Tandon states that the "dorsal vessel" is double. This De Quatrefages denies, but Moquin-Tandon was right. The dorsal vessel generally lies in the dorsal sinus, but comes outside it more often than in *Pontobdella*. De Quatrefages states that the ventral vessel is double. The ventral vessel lies for the greater part of its length outside the ventral sinus.



The supernumerary vessel of De Quatrefages is in reality the ventral vessel. The only point concerning which I am able to speak from my own observations is with regard to the relations of the vascular dilatations in the branchiæ.

These dilatations occur in the first pair of branchiæ, and in every third succeeding pair.

Branches of the lateral vessel run into these and end by an open mouth, the dilatation on the other hand communicates with the lateral sinus which runs parallel to the lateral vessel.

It seems to me quite clear that these branchial vascular dilatations of Branchellion are represented by the rudimentary dilatations described above in Pontobdella, Piscicola, and Clepsine, and that probably we have here the only communications between the "closed" vascular system and the system of lacunar spaces or sinuses.

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It is unnecessary for me to recapitulate here the condition of either system in these four genera, they present a marked unity of type, all approaching more or less closely a condition in which four longitudinal trunks communicate with one another and lie respectively in four sinuses which fully communicate, and which, or their branches, contain several of the principal organs. We have, in fact, a vascular system on the one hand, and a cœlomic system upon the other, branches of the former opening into the dilatations of the latter in the lateral region in the skin, thus establishing a direct continuity of the lumen throughout.

#### GNATHOBDELLIDÆ.

How slightly, but how regularly, the Gnathobdellidæ vary from the Rhynchobdellidæ in this respect we shall now see. The differences may be briefly summed up thus:

1. In the Gnathobdellidæ all trace of lateral sinus and of the dilatations connected with it has vanished.
2. All trace of the dorsal and ventral vessels has vanished.
3. The lateral vessels with their connections and the dorsal and ventral sinus system are placed in communication only

through a new development, viz. botryoidal tissue, which may play an important rôle, forming a secondary cœlom.

### Hirudo.

The Lateral (longitudinal) Vessels.—These vessels anastomose with one another anteriorly and posteriorly (fig. 5, *l. v.*).

They give rise to two sets of branches; the one set arise from the internal border, the other from the external, the former divide almost immediately into two diverging branches which anastomosing—those of the one side with those of the other—upon the abdominal wall, beneath the ventral vessel, establish along the whole length of the body a direct communication between the two lateral vessels; the trunks giving rise to these anastomoses are eighteen in number on each side, and have been called by Dugès latero-abdominal branches (fig. 5, *l. ab.*).

The branches arising from the external borders of the lateral vessels are alternately long and short; the short are distributed to the median lateral region, and may be called latero-lateral branches (*l. l.*). The long branches (*l. d.*, latero-dorsal, Dugès) passing dorsalwards divide into two branches which both approach the median dorsal line, but never have, as Cuvier pointed out, any direct connection with the dorsal vessel (sinus A. G. B.); of the two branches one is anterior, the other posterior.

Those branches of the latero-dorsal vessels, situated anteriorly to the gastro-ileal region, never anastomose from right to left side.

In the gastro-ileal region the posterior branches of the latero-dorsal vessels follow the common law, but not so the anterior branches; these latter anastomose, those of one side with those of the other, forming arches above the gastro-ileal intestine.

All these branches resemble the lateral vessels in the muscular structure of their walls.

Cutaneous Networks.—These originate from the above-mentioned branches, and form three successive layers (the

most superficial of these alone deserves the name cutaneous in that sense of the term which I have adopted).

1. The Deep Layer is divided into four longitudinal bands, two of these are situated upon the dorsal surface—the larger two. These arise from the latero-lateral and latero-dorsal vessels. The other two bands are smaller. They are situated on the ventral surface right and left of the ventral sinus; they arise from the latero-abdominal vessels. These capillary networks are formed of botryoidal tissue (*réseaux variqueux*, Gratiolet). They arise from the branches which are going to the skin.

2. The Intermediate Layer.—The vessels composing this network arise from branches coming directly from the lateral system, and communicate on the other hand with the botryoidal tissue. They form, by anastomosis among themselves, superposed networks which become finer and finer as they near the surface, and finally passing into the subepidermic region give rise to :

3. The Superficial Layer.—The vessels forming which are extremely fine and form an almost complete network; it is these vessels which penetrate the epidermic layer.<sup>1</sup>

In the lateral regions small vessels, capillary at both extremities, run in a vertical direction connecting the dorsal and ventral superficial networks; they may be termed vertical superficial branches (Gratiolet).

These superficial networks are developed in a bilateral manner, the communications between those of one side and those of the other being few in number and excessively fine in calibre.

Ramifications of the Latero-abdominal Vessels.—These latero-abdominal vessels, as described above, divide into two diverging branches, an anterior and a posterior.

The main trunk of the latero-abdominal furnishes a vessel which ascends along the anterior limb of the “main lobe” of the nephridium:<sup>2</sup> the vesicle of the nephridium receives its vessels from its posterior bifurcation.

<sup>1</sup> Lankester, ‘Quart. Journ. Micr. Sci.,’ 1880, p. 303.

<sup>2</sup> Bourne, ‘Quart. Journ. Micr. Sci.,’ 1880, Pl. XXIV.

These vessels going to the vesicle of the nephridium have two origins; the first branch passes directly to its more anterior portion, the second bifurcates, one portion ramifying upon the internal portion of the vesicular wall, the other passing ventrally becomes connected with a small botryoidal network, but terminates by anastomosing with the anterior branch of the next following latero-abdominal vessel.

These two vessels form a rich network upon the walls of the vesicle, giving rise to a small trunk, which, passing forwards parallel to the vesicle duct, breaks up into capillaries upon the posterior limb of the main lobe of the nephridium (for the arrangement of these and all the nephridial vessels, see my figures of the nephridium, loc. cit.).

The nephridium thus receives a double blood supply from the lateral vessel, a direct supply anteriorly, coming from the latero-abdominal vessel, and an indirect, posteriorly, arising from the capillary network of the vesicular wall.

The latero-abdominal branches also supply, according to Gratiolet, the epididymis, the ovaries, and the copulatory organs, male and female, but not the testes (see below).

The Capillary System of the Nephridia communicates on the one hand with the "cutaneous" dorsal networks, on the other with the ventral sinus.

The first communication is established by tortuous ramifications collecting upon the outer (dorsal) border of the main lobe and running dorsally to anastomose with any of the three "cutaneous" dorsal networks.

The second communication is established by an important system of vessels described for the first time by Gratiolet; these arise between the two portions of the main lobe of the nephridium, and, receiving branches from the apical lobe and testicular lobe, pass alongside the latter on to the surface of the testis and dilate there, forming the so-called "moniliform hearts" described by Brandt.

It is in these dilatations that the funnel of the nephridium of *Hirudo*, which I shall describe below, and which has escaped the notice of all previous observers, is lodged.



These dilatations, says Gratiolet, do not exist in connection with the nephridia anterior to the first testis, but behind this point all the nephridia have them, and depending upon or rather determining this arrangement is the fact that the four anterior nephridia do not possess funnels, while the others do, even the two which are placed behind the testicular region.

These "perinephrostomial sinuses," as I propose to call them, which are in *Hirudo* merely special developments of the circumtesticular sinuses of *Pontobdella* (see p. 456) are severally in direct communication by means of a short vessel with the ventral sinus, also from each passes a vessel which ascending, ramifies among the dorsal networks, the cardio-dorsal branch of Gratiolet.

In addition to these arise the capillary networks upon the testicular walls. These networks represent in fact a circumtesticular sinus which has become reduced by diacœlosis (woodcut, fig. 4, *te.*).

The capillary networks upon the testicular walls are connected with the botryoidal and other networks in the ventral region.

**The Ventral Sinus.**—The ventral sinus was first recognised as a blood space containing the nerve-cords by Johnson.<sup>1</sup> In the region of the nerve-ganglia sinuses are connected with the ventral sinus on either side; these are connected with the capillary networks of the dorsal region; they necessarily pass close to the walls of the crop. In the gastro-ilcal region these pass with the cardio-dorsal trunks at the sides of the intestine between this and the last pair of cæcal dilatations of the crop. They have been termed by Dugès abdomino-dorsal branches (sinuses, A. G. B.). These, together with the cardio-dorsal trunks and the short vessels of Brandt, which occur in the interval between two pairs of the abdomino-dorsal sinuses, like the vertical superficial branches, connect the dorsal and ventral networks. Thus the three systems of dorso-ventral trunks are the sole origin of the vascular supply of the walls of the crop, this latter representing the primitive peri-

<sup>1</sup> 'A Treatise on the Medicinal Leech,' 1816, p. 115.

enteric sinus which is but slightly broken up in the Rhynchobdellidæ.

As might be expected from the absence of the perinephrostomial sinuses, the cardio-dorsal branches are absent anteriorly to the first testes.

**The Vessels of the Gastro-ileal Intestine, and of the Dorsal Sinus.**—The vessels of this portion of the alimentary canal arise exclusively from the five large anastomotic arches which are formed above it by the anterior branches of the latero-dorsal vessels. Each of these furnishes two trunks which descend parallel to one another, the one on the right, the other on the left side of the intestine, and each terminates by forming a nearly rectilinear longitudinal vessel; these vessels occurring on either side of the intestine give numerous branches to its walls. They may be termed collateral arteries. Their branches penetrate the intestinal wall, ramify in the spiral valve, and give rise to a vessel which runs along the whole length of the free edge of the valve.

The capillaries of this vessel (marginal vessel of the spiral valve, Gratiolet) open into two median longitudinal sinuses, situated, the one immediately above, the other immediately below the intestine.

The inferior of these communicates with the superior in front of the gastric dilatation by two symmetrical branches, which surround the strangulated portion of the canal which terminates the crop in front of the stomach.

The superior of these two, opening in front into the dorsal sinus, divides posteriorly into two branches, which, passing down at the sides of the rectum, open into the dilatation of the ventral sinus, which lodges the last ganglion of the nerve cords.

Thus a direct communication is established between the dorsal and ventral sinuses.

**The Dorsal Sinus** (fig. 62, and woodcut, fig. 4, *d. s.*).—This sinus extends above the alimentary canal, along the entire length of the body. It has no communication with the ventral sinus by means of the abdomino-dorsal branches of the latter.

The dorsal sinus communicates directly with the ventral sinus only at its posterior extremity, as described above.

No branches are supplied to the crop, but considerable branches are given off at intervals—two in each somite, in the region where they exist, thirty-two pairs in all—and passing through the botryoidal tissue terminate by anastomosing with the superficial cutaneous networks.

Such is the complicated system of vascular spaces in *Hirudo*.

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The anatomical relations described above, which possess the greatest interest and importance for the solution of the problem : How far are these spaces “cœlomic” in nature? may be summed up thus :

The whole system of vessels and sinuses is in continuity.

The lateral vessels communicate freely with one another without the intervention of any capillary system ;

They possess branches opening into botryoidal or other capillary networks of the “cutaneous” system ;

They also form nephridial capillaries, which are partly collected again and carried to the capillaries of the “cutaneous” system, and partly unite to form a vessel which is connected with the perinephrostomial sinus ;

Again, they form capillaries upon the intestinal wall.

The dorsal sinus is directly connected with the ventral sinus.

The dorsal and ventral sinuses also severally communicate with—

1. The cutaneous networks ;
2. The capillary network upon the walls of the crop ;
3. The capillaries upon the intestinal wall and upon its spiral valve ;
4. The perinephrostomial sinuses.

The botryoidal and other “cutaneous” capillary networks, which appear to be one and the same system, communicate, on the one hand, with branches of the lateral vessel, and upon the other with the extensions of the dorsal and ventral sinuses.

The capillaries upon the wall of the crop are solely developments of these extensions.

The vessels of the walls of the gastro-ileal tube, the true digestive portion of the canal, are directly derived from branches of the lateral longitudinal vessels.

The ventral sinus contains the nerve chain.

The perinephrostomial sinus contains the nephridial funnel.

The network of capillaries upon the testicular wall potentially contain the testis.

We now turn to the histological side of the question.

The lateral vessels and their branches possess a very definite muscular wall; the smaller branches and capillaries only, have no muscular development.

The dorsal and ventral sinuses and their extensions never possess muscular development, their walls consisting of connective tissue; the larger of them, the ventral sinus especially, may exhibit a large amount of pigmented tissue in the wall.

The botryoidal tissue is in reality composed of intracellular tubes.

The vaso-fibrous tissue is of the same nature, and it is such tissue which forms the intermediate "cutaneous" network, i. e. that which lies among the muscular tissue.

The vaso-fibrous tissue and the botryoidal tissue are developed from pigmented connective-tissue corpuscles and fibres, the central portion of which liquefies, forming blood, the nuclei forming corpuscles, the walls thinning out and forming thin-walled capillaries, or it may simply become hollow, and allow the blood in pre-existing capillaries to flow into them.

Considering this double series of facts, it seems to me permissible to draw the following conclusions:

The ventral and dorsal sinus and the extensions in connection with these, either containing organs, e. g. the nerve-cord and the nephridial funnels, or forming a complete network around organs, correspond to a surrounding sinus in other genera, and represent "cœlom."

The lateral vessels and branches represent a closed vascular



system, but this has not become quite closed, and remains in connection with the cœlom.

The botryoidal and other capillaries present considerable difficulty, and their consideration is best deferred until after an examination of the other genera of the Gnathobdellidæ.

Two other liquid-holding spaces occur in *Hirudo*, which, I think, possess great interest.

The ovaries are filamentous bodies with a club-shaped termination; the round bodies seen in connection with the oviducts, and usually termed ovaries, are in reality merely sacs containing the true ovaries, and also a fluid devoid of hæmoglobin, but containing amœboid corpuscles. What are these corpuscles? I regard them as primitive blood-corpuscles, similar to those found in the Rhyncobdellidæ, and although, of course, until we have some direct observations made upon the embryo, it is a mere conjecture, I expect that the oviducts possess at one period open mouths, opening into a cœlomic space, which contains amœboid corpuscles, and that they close around the ovaries before hæmoglobin is developed. This theory receives great support from the fact that in *Clepsine*, *Pontobdella*, and *Branchellion*, the ovaries lie in the ventral sinus, which contains, in common with all the blood-spaces in these genera, amœboid corpuscles.

I have not at present specially studied the generative organs in the group, but I think that even in the genera above mentioned the oviducts have acquired a connection with ovary, and that having primitively open mouths similar to those of the *Chætopoda*, these have become closed around the ovary.

The other space in *Hirudo* which it seems to me may have a similar morphological significance, is a sinus around the *vas deferens*.

In *Pontobdella* the *vas deferens* is an exceedingly fine canal with ciliated walls. In *Hirudo* it is apparently of considerable size, but on further examination the actual canal is found to be exceedingly narrow and a sinus is found around it packed with cells which possess rather a degenerate appearance but are very similar to the amœboid cells found in the periovarian sinus. No sinus

remains developed around the vas deferens in *Pontobdella*, but around the testes there is an almost complete sinus as described above. Taking these facts into consideration it seems to be quite possible that we have in these perigenital spaces portions of coelom shut off at a very early period and in the amœboid cells and colourless fluid—the primitive blood of *Hirudo*.

#### *Hæmopis*, *Hæmadipsa* and *Aulostoma*.

I have little to add regarding the vascular system in these genera to what has been already described in *Hirudo*.

In *Hæmopis*, the dorsal sinus appears to be much less developed than in *Hirudo*.

In *Aulostoma* the dorsal sinus has ceased to exist.

In *Aulostoma* (a fact which lends great support to the theory enunciated above) the spaces surrounding the female generative organs contain a red fluid.

#### *Nephelis* and *Trocheta*.

These genera present some most important variations upon the arrangement described in *Hirudo*. (See woodcut, fig. 5, p. 456.)

The lateral vessels and their branches, in so far as I have traced them, present a very similar distribution. The ventral sinus is exceedingly large but the dorsal sinus is entirely absent (fig. 63).

It is in the botryoidal tissue that we find the most remarkable new development.

The botryoidal tissue is arranged as in *Hirudo* in dorsal and ventral longitudinal bands which communicate (those of the one side with those of the other) much more freely than in the former genus.

The botryoidal tissue exhibits throughout the body a tendency to acquire a very large lumen and appears to be capable of great distension by blood. In the lateral regions of the body occurs a metameric series (11 pairs) of dilatations of botryoidal tissue (fig. 62, *cœl.*). They only occur in the central portion of the body, the most anterior pair being posterior to the copulatory organs.

When empty of blood they shrink in virtue of a slight muscular development in their walls to a very small size, but when fully distended each presents a diameter of perhaps a quarter the breadth of the body. They are about twice as long as broad and each presents a constriction in the centre, being divisible into an anterior and a posterior portion of nearly equal size. Within these are lodged the funnels of the nephridia, (figs. 51 and 62).

They have been long known in *Nephelis*, having been first described by Johannes Müller under the name of Lungengefässen, but although functionally they are perinephrostomial yet they differ morphologically from the perinephrostomial sinuses of other *Hirudinea*. And I think it would be well to insist upon their histological significance by terming them botryoidal sinuses.

They have been described by Von Siebold<sup>1</sup> and Leydig,<sup>2</sup> but more recently and fully by Bidder,<sup>3</sup> the latter observer showing that they contained the nephridial funnels which were discovered by Von Siebold.

In *Nephelis* they are mere enlargements of the lumen of the botryoidal tissue in the neighbourhood, this latter forming a dense mass around them, a very slight muscular development occurring in connection with their walls and serving to empty their contents.

In *Trocheta*, actually bounding the lumen, there is a single layer of cells containing pigment granules, and presenting a slightly ragged inner surface; these are clearly cells similar to those forming botryoidal vessels (fig. 51, *ep.*). A definite muscular wall obtains, the muscle-fibres themselves being very small; outside this there is a considerable amount of ordinary ectoplastic connective tissue.

The lumen is thus not continuous on all sides with that of the botryoidal tissue around it as in *Nephelis*, but there is a definite vessel opening into the sinus (fig. 51, *t*) which has the

<sup>1</sup> 'Lehrbuch der vergl. Anat.,' p. 216.

<sup>2</sup> 'Berichte von der König. zootom. Anstalt zu Würzburg,' 1849, p. 14.

<sup>3</sup> 'Untersuch. einiger Hirudineen,' Dorpat, 1868.

nature of a cæcal diverticulum formed as a special development of botryoidal tissue. The lumen in both genera is in direct communication with the ventral sinus through the intervention of botryoidal tissue, and with the lateral vessels of its own side through the intervention of the branches of the latter, which terminating as capillaries, open into the botryoidal tissue.

General conclusions with regard to the cœlom in the Hirudinea.

The somewhat scanty embryological evidence which we possess upon this point favours the view that the cœlom develops by a splitting in the mesoblast, that it is in fact that modification of an enterocœle which Huxley has termed a schizocœle.

This cavity persists to some extent in all the genera, and while it remains most fully developed in the Rhynchobdellidæ, it is reduced to a minimum in Nephelis and Trocheta, being there represented only by the ventral sinus and its immediate branches.

In the Rhynchobdellidæ, at any rate in Clepsine, Pontobdella and Branchellion, the cœlomic remnants (sinuses) continue to be lined with cœlomic epithelium cells. These cells (figs. 41—43) are exactly similar in all three genera (I have not looked for them in Piscicola). In many places they form a continuous layer, but most generally some of these have come free, sometimes almost all, and are to be seen floating in the blood. These free cœlomic-epithelium cells are only to be seen in the sinuses, they are probably too large to pass through the communicating channels. In the Gnathobdellidæ there is no trace of such cells.

A process has been taking place which I propose to term diacœlosis, a "scattering" of the cœlom, connective-tissue growths having more or less completely filled it up, the remnants forming the sinus system.

Different remnants remain in different genera. The organs which in animals possessing a well-developed cœlom lie within that cœlom, either get blocked out by connective-tissue growth, or remain enclosed in the remnants. The same organs may



remain in different remnants in different genera. No better instance can be given of this than the varying position of the nephridial funnel in *Clepsine*, *Pontobdella* and *Hirudo*.

The lumen of existing cœlom as above described comes into communication with the lumen of a true vascular system which was probably derived at a very early period from the archaic enterocœle. That such communication is of a secondary nature and not a persistence of the original communication, which must have existed if one developed from the other, is indicated by the existence of the colourless amœboid cells in the ovarian sac, and around the vas deferens in *Hirudo*. These were probably closed at a very early period before the development of hæmoglobin. This may have a phylogenetic bearing only, but it may be, and I should think very possibly is, a process which is repeated ontogenetically.

The communication between existing cœlom and the true vascular system occurs in one of two ways:

1. Vessels may terminate with an open mouth, which is apparently provided with a sphincter in certain portions of the cœlom, e.g. the lateral dilatations and branchiæ.

2. Vessels (capillaries only, probably) may acquire a connection with new sp<sup>3</sup> (botryoidal, &c., tissue), which are forming in the connective tissue, these communicating on the other hand with small cœlomic remnants.

The former of these ways is characteristic of the *Rhyncobdellidæ*, the latter of the *Gnathobdellidæ*.

This development of new cœlomic space (botryoidal tissue) may be termed metacœlosis.

This new space in its highest development encloses the nephridial funnel (*Nephelis*), and the perinephrostomatous portions of it may acquire a definite musculature, and the "botryoidal" cells become modified, so as to form a secondary cœlomic epithelium, as in *Trocheta*.

An archaic enterocœle thus gradually undergoes diacœlosis, being replaced by a metacœle. This primary and secondary cœlom occur simultaneously side by side in all existing *Gnathobdellidæ*. In the *Rhyncobdellidæ* considerably more of the

primary cœlom remains, and the secondary cœlom has not yet appeared upon the scene.

#### vii. NEPHRIDIA.

Since the appearance of my memoir on the nephridium in *Hirudo*,<sup>1</sup> where a full account of the previous literature is given, Arnold Lang<sup>2</sup> and Oscar Schultze<sup>3</sup> have dealt with this subject, and in a second note<sup>4</sup> I have demonstrated the existence of proper walls to the so-called central duct, the lumen being intracellular. The importance of this fact will be shown below. Schultze's paper forms a most valuable addition to our knowledge, correcting my previous observations, and, as I have since convinced myself, rightly in some important particulars. Schultze also deals with *Clepsine*, *Nephelis*, and *Aulostoma*, and his results agree in the main with my own independent observations; these latter, especially with regard to *Pontobdella*, to the existence of an internal funnel in *Hirudo*, and to the above-mentioned fact regarding the nature of the so-called central ducts, enable me to give a connected description of the nephridia in the group of the Hirudinea.

#### *Pontobdella*.

Some of the nephridial funnels in this genus were described and figured by Vaillant, but the network of tubules has hitherto entirely escaped notice. Since the publication of a note on this subject by the Royal Society<sup>5</sup> I have spent several months at Naples working at this subject, and the results I then obtained are here incorporated with my previous results.

The nephridium in *Pontobdella* is without doubt the most

<sup>1</sup> 'Quart. Journ. Micr. Sci.,' 1880.

<sup>2</sup> 'Mitthl. Zool. Stat. Neapel.,' 1881.

<sup>3</sup> 'Arch. f. mikr. Anat.,' 1883.

<sup>4</sup> 'Quart. Journ. Micr. Sci.,' 1882.

<sup>5</sup> 'Proc. Roy. Soc.,' 1883.

remarkable structure in the whole group. It is a single continuous organ, a most marvellously complicated network of tubules, communicating those on one side of the body with those on the other, and continuous throughout the greater portion of the body.

The tubules lie inside the muscular layers of the body wall and among the connective tissue and gland cells. They extend as far forwards as the ninth nerve-ganglion, and as far backwards as the nineteenth (figs. 3 and 8).

**Internal Funnels.**—There is a paired series of funnels lying in the ten segments, 9 to 18 inclusive. These funnels lie in the dorso-ventral blood sinuses (fig. 8, *neph. fun.*). The funnel itself is composed of from four to six rounded cells with large nuclei; these cells are richly ciliated upon their free surfaces, and surround the lumen (figs. 53, 54). The lumen of the funnel often appears, however, to be occluded, its place being occupied by a mass of vacuolated protoplasm (fig. 54, *b.*); the funnel does not probably possess much functional activity. This occluding of the lumen is, as we shall see, carried to a much greater extent in *Hirudo*. Following upon the funnel itself is a short neck (fig. 54, *a.*), presenting a large nucleus in the wall and a ciliated lumen. This opens into a considerable dilatation; the walls of this dilatation consist of numerous flattened cells, and appear, together with the neck, to be partially covered by an epithelium (*l. c., ep.*).

I have traced the communication of this dilatation with the tubules in sections; it may, moreover, be sometimes seen in fresh preparations (fig. 53, *x.*).

The dilatation has very curious contents, and appears at times very much distended. As stated above, and as may be seen from figs. 53 and 54, the whole apparatus, funnel and dilatation, lies in a blood-sinus, in which float freely, blood-corpuscles and cœlomic epithelium-cells, and the ciliary current carries corpuscles down the funnel from time to time. These cannot apparently pass further, and lodge in the dilatation.

The contents seem to consist mainly of degenerating or

macerating corpuscles; there are, in addition, a large number of very fine filaments, which tend to have a radiating arrangement with regard to clumps of corpuscles, or sometimes to bind them together. These may be observed continually moving to and fro, a movement caused, I believe, by the ciliary current, and not in any way intrinsic. The filaments present an even diameter, are exceedingly fine, and sometimes much twisted; they readily stain with iodine. They are shown as seen with Hartnack's No. xii à immersion in fig. 55. I think these filaments are most probably somewhat of the nature of fibrin, and are formed from time to time in the blood in abnormal conditions. The possibility of their being leptothrix filaments entered my mind, but they are always present, and no other bacteria forms are ever visible, both of which facts militate strongly against this view. I have noticed somewhat similar filaments in the dilatations following upon the funnels in *Nephelis*, *Clepsine*, and *Hirudo*, although nowhere are they developed to the extent that they are in *Pontobdella*.

Network of Tubules.—The tubules consist of simple or branched cells with an intracellular lumen.

In the fresh state the wall often presents surface markings similar to those I have described in *Hirudo* (l. c.); these are shown in fig. 56. In optical section the walls of the lumen exhibit a radiating structure (see figs. 57, 58, &c.). The cells attain a great size, fig. 58 showing a single cell—a cell containing caecal diverticula of the lumen. In some places the wall of the lumen is thrown up into little vesicles, generally arranged in groups. At such places the wall becomes much thinned out (fig. 57), and the lumen must come into very close physiological relations with its surroundings.

The lumen may vary in extent from spot to spot, and from time to time, the walls being capable of distension. Fig. 60 shows the appearance of a portion of the network in the fresh condition. Fig. 59 shows a portion in a preparation which has been macerated in 10 per cent. nitric acid. An examination of this figure will show how few nuclei occur, and consequently how large and much branched must individual



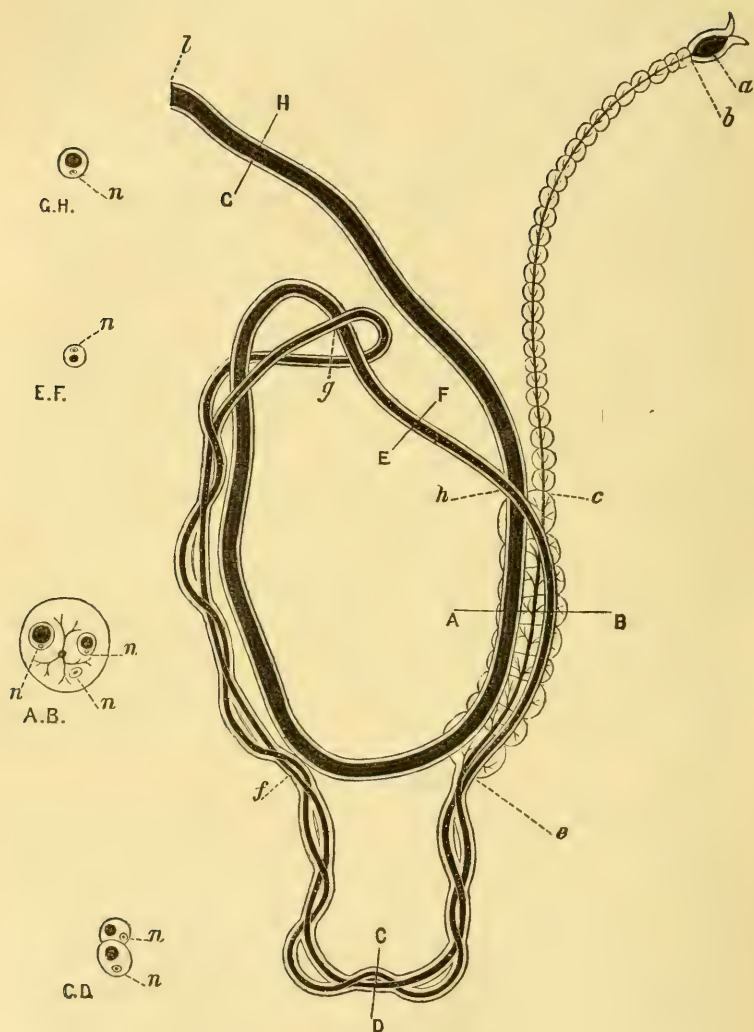
cells become; it also shows the relation of the nephridial network to the capillary network. By macerating a whole fresh *Pontobdella* in nitric acid (10 per cent.), after cutting it open and clearing away the alimentary canal, I have been enabled to remove from the outside, first, the epidermis and then, the muscular layers. By proceeding in this manner with exceeding care, taking the muscles away a few cells at a time, the nephridial network, extending over two or three segments, may be left in a thin layer, containing, in addition, the capillaries and gland-cells and connective tissue, and a few muscle-fibres helping to hold it together. By such preparations, which at times are very successful, I have been enabled to construct the diagrammatic drawing in fig. 8.

**Apertures to the Exterior.**—At regular intervals, as shown in fig. 3, the tubules collect together: the lumina become much larger, and then communicate with a duct leading to the exterior (fig. 65, *neph. apert.*). This duct is lined by numerous small cells, resembling ordinary epidermic cells, and has the appearance of being invaginated from the exterior; it is, indeed, lined by a cuticle. There is no vesicle or dilatation corresponding to the vesicle of other genera (fig. 65).

These paired apertures are situated upon the anterior and outer surface of the second papilla (counting from the median ventral line) of the first annulus in the ten segments, 10 to 19 inclusive (fig. 3).

### Branchellion.

I have not had sufficient suitable material to be able to speak with absolute certainty regarding Branchellion. In one instance I obtained a living specimen small enough to enable me, by very gradually increasing compression, such as adopted by Fraipont and Lang, in examining the nephridia in Cestodes, Trematodes, and Planarians, to observe the nephridial network. It is a perfectly continuous network, occupying the same region of the body as in *Pontobdella*; the tubules present a similar structure, but in this young specimen, at any rate, I could only detect a single pair of



**FIG. 6.**—Clepsine. Diagram of a nephridium. *a*. Funnel. *b*—*c*. Testis lobe. *c*—*e*. Main lobe. From *e*, through *f*, to the apex *g*, and back through *f* to *e*, runs the recurrent duct; this then passes through the main lobe to *h*, where it runs across to the apex *g*. *g*—*f* corresponds to the apical lobe of *Hirudo*: from *f* the duct runs across to *e*, where it re-enters the main lobe; at *h*, it emerges and passes as efferent duct to *l*, the external aperture.

*AB*, *CD*, *EF* and *GH*. Sections through the regions so marked. *n*, *n*. Nuclei. The section *CD* is taken a little to the right hand of the line *CD*.

Section *AB* is a cell of the main lobe with its nucleus *n*, and contains branching ductules; and in addition to these, it surrounds in two places perforated cells containing the duct in two subsequent portions of its course, also showing nuclei, *n*, *n*, in their walls.

Section *CD* shows two perforated cells in juxtaposition, each containing a portion of the recurrent duct; the one as it goes to, the other as it comes from, the apex.

Section *EF* shows the single perforated cell which contains the duct in that portion of its course.

Section *GH* shows the similar, but larger cell containing the efferent duct.

FIG. 7.—*Hirudo*. Diagram of a nephridium. *a*. Funnel. *b—c*. Testis lobe. *c—d*. Main lobe. *e—d*. That portion of the main lobe not represented in *Clepsine*. *e*. Point where the recurrent ductules leave the main lobe on their way to the apex *g*, and where the recurrent duct re-enters the main lobe when returning from the apex *g*. *e—f*. Recurrent lobe; for the remainder of their course the recurrent ductules and duct traverse the apical lobe, *f—g*. *g*. Apex and point where the recurrent ductules collect to form the recurrent duct; this latter, as in *Clepsine*, returns to *e*, re-enters there the main lobe, emerges thence at *h* to pass across to the apex, and traversing the apical lobe returns to *f*, where it passes across into the free end of the main lobe *d*; it emerges from the main lobe at *h*. *h—j*. the vesicle duct (efferent duct). *j—k*. The vesicle. *l*. The aperture to the exterior.

A B, C D, E F, G H. Sections through the regions so marked. *n, n*. Nuclei.

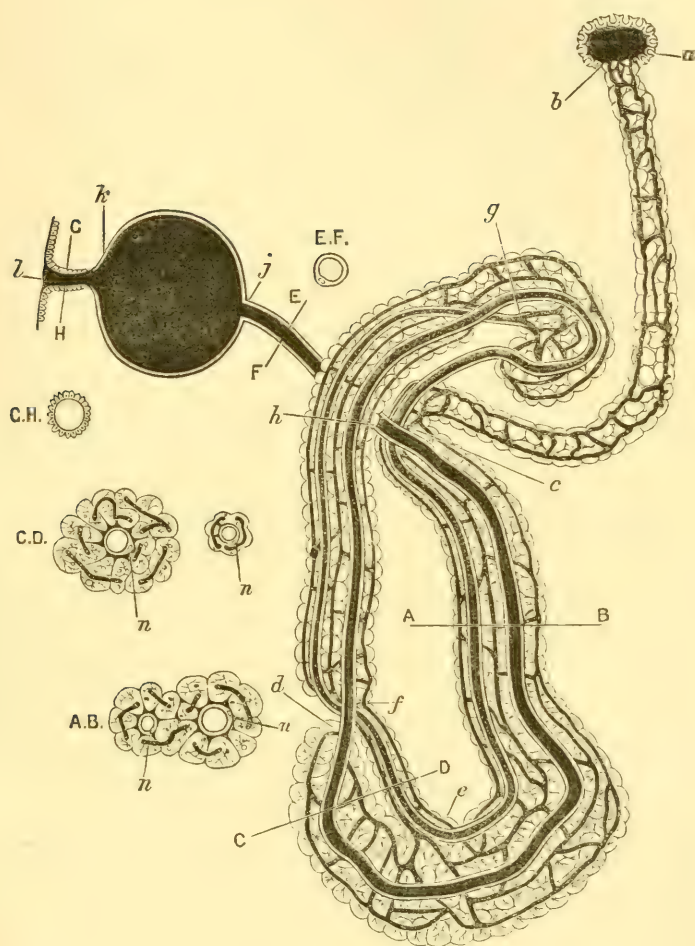
Section A B shows the cells of the main lobe, with their branching ductules; the nuclei of these cells are drawn but not lettered. The cells are grouped around two perforated cells, which contain the "recurrent" and "main" portions of the duct. *n, n*. Nuclei in the walls of these perforated cells.

Section C D passes through the cœcal end of the main lobe, and also through the recurrent lobe. The main lobe section shows similar arrangements to those shown in the Section A B. The section through the recurrent lobe shows the recurrent ductules branching in the cells, which are grouped around the perforated cell containing the recurrent duct.

Section E F passes through one of the perforated cells containing the vesicle duct.

Section G H passes through the numerous cells bounding the duct in the last portion of its course, and lined internally by a prolongation of the cuticle from the body surface.





external apertures, and these correspond in position to the most posteriorly placed pair of apertures in *Pontobdella*. Not only here, but after much careful searching through several complete series of sections of *Branchellion*, taken in various planes, I have been unable to find any funnels; indeed, I am almost certain that none such exist. *Branchellion* would thus present a more archaic form of nephridium than even *Pontobdella*, and on this account is extremely interesting.

#### *Piscicola*.

I have seen similar tubules in sections, and it is not difficult to observe the external nephridial apertures in this genus; ten pairs may be counted.

#### *Clepsine*.

I have very little to add to Oscar Schultze's account of the nephridia in this genus. I have found it perfectly easy to trace the funnels in my sections: these open into the ventral sinus (fig. 52). Following upon the neck of the funnel is a dilatation corresponding to that described above in *Pontobdella*, and as in the latter genus it becomes packed with corpuscles. Following upon this (corresponding to the "testis lobe" in *Hirudo*) is a single row of cells (woodcut, fig. 6, *b.c.*) in which the lumen is only very feebly developed, the lumen then enters the series of cells *c—e*, branching in the cells (see woodcut, fig. 6, *A B*). The cut also shows how these cells are penetrated in two places by the duct in its subsequent course.

At *e* the lumen enters a series of very much elongated cells, it passes onwards to what corresponds to the apical lobe in *Hirudo* (*g.*) and then returns upon itself, perforating another series of elongated cells and returning to *e*, where with its own special walls it passes through the series of rounded cells, *e—c*. The section *c. d.* shows the two lumina each perforating its own cell as they exist in the region *e—f*. At *c* the duct, perforating another series of elongated cells as shown in the section *E F*, passes down to the apical lobe and, perforating a single series of cells, runs to *f*, across to *e* and once more with its own cell-

constituted walls passes through the rounded cells *e—c* and then emerging at *h*, perforates a single series of cells *h—l*, and passes down to the external aperture *l*. The section *AB* shows the condition of one of the rounded cells which thus has its own lumen receiving ductules and is perforated in two places by cells each with its own special lumen. This gives the cell a most remarkable histological character.

The lumen of the nephridium in *Clepsine* might be actually dissected out, as is represented diagrammatically in woodcut, fig. 8, *c*. There is the funnel *a*. Upon this follows a portion *b—c*, with a very slightly branched lumen and then a portion, *c—e*, with a rather more branched lumen, but from *e* to the external aperture the duct is perfectly simple, always perforating single cells, and without branches.

#### Gnathobdellidæ. *Hirudo*.

The funnel in *Hirudo* has hitherto entirely escaped observation and its existence was denied by myself, by Schultze, and by all previous observers.

I have already fully described its position—it lies in a special blood sinus, surrounded by red blood. This is the perinephrostomial sinus (fig. 61, *pn. s.*; woodcut, fig. 4), and in those segments containing testes (7—16) lies upon the dorsal wall of the testis. In the two segments, 17 and 18, which contain nephridia posterior to the testicular region, the funnels exist in similar sinuses, while in the five nephridial bearing segments (2—6) anterior to the testicular region the funnels do not exist. The funnel lies at the end of the “testis lobe.” This testis lobe ends in a mass of cells of a spongy nature (fig. 49, *b.*), the ductules being very irregularly arranged. The arrangement and the character of the funnel about to be described seem to me to be explicable by a theory of degeneration following upon a loss of function. Attached to the extremity of the “testis lobe” is a large mass covered with cells, which generally present upon their free surface a somewhat bilobed appearance due to a depression upon the surface; such cells have the shape shown in fig. 50, *B*, others of these cells are

merely rounded (l. c., c.) and others much flattened (l. c., A.), they present a nucleus (*n.*) and very often a lumen (*l.*); these cells represent either separate funnels or more probably lobes of a simpler funnel. They are set upon the walls of a dilatation corresponding to the dilatations in *Clepsine* and *Pontobdella*, and as in those genera containing a sort of *débris*. In *Clepsine* we have seen a very simple funnel to be present, two cells taking part in its formation, in *Pontobdella* more cells take part in its formation, and it becomes somewhat lobed, and in *Nephelis* and *Trocheta* this is carried to a still greater extent, and it seems very probable that the structure found in *Hirudo* is the same thing as the funnels of the other genera, only that this division into lobes has been carried to the extreme, and at the same time, although it is a point exceedingly difficult of determination, the communication between the lumen of the tubules and the *cœlomic sinus* seems to have been obliterated. It is interesting to note in this connection that the nephridium of *Hirudo* possesses a much fuller blood-supply than the nephridium of the genera which present a better developed funnel.

Following upon the funnel is the "testis lobe" (woodcut, fig. 7, *b—c*). This is throughout a spongy mass, the ductules being very irregularly arranged. At *c* the cells of the "testis lobe" are continuous with those of the "main lobe" (*c—d*). It is in these cells that the ductules become so remarkably branched, the main ductule receiving collecting ductules (Cf. my figure in 'Quart. Journ. Micr. Sci.,' vol. xx, Pl. XXIV, fig. 5). The ductules collect together, those from the terminal portion (*d—e*) as well as those from the main portion (*c—e*), at *e*; the lumen simply perforates the three or four rows of cells which constitute the portion *e—f*.<sup>1</sup> At *f* these cells join

<sup>1</sup> The term "recurrent lobe," which I proposed for this portion, may still be used, for although we now know the lumen to be simply continued from funnel to external aperture without any recurrent portion in the sense in which I previously used the term, yet the ductules in question pass from *e*, to the apex *g*, and then return to *e*. I now speak of the lobe *e—f*, as the "recurrent lobe;" the ductules running from *e* to *g*, as the "recurrent ductules," and the portion of the duct returning from *g* to *e* as the "recurrent duct."



the cells of the "apical lobe;" the lumen here passes through the "apical lobe" to *g* as a network without receiving any collecting ductules, and at *g*, which may be termed the "apex," the lumen becomes single, and from that point to where it enters the vesicle simply perforates a series of drain-pipe cells (fig. 48, *d*, woodcut, fig. 7).

The duct in this condition—i. e. with its own special walls—passes back through the "apical lobe" lying between the other cells (woodcut, fig. 7), through in the same manner the portion *f*—*e*, through the "main lobe" from *e* to *h*, where it emerges to penetrate the "apical lobe" once more, and, passing back past *f*, enters the end of the "main lobe" at *d*. It now traverses the main lobe to *h*, where it emerges again to pass to the vesicle (*j*), being in its whole course from the funnel up to *j* an intracellular duct. In my previous paper I described a portion as a "central duct"—an intercellular portion lying between the cells in the lobes which it traverses—but the discovery<sup>1</sup> of the nucleated protoplasmic walls (fig. 48) of this portion of the duct—in other words the discovery, that it was an intracellular and not an intercellular duct—necessitates the abandonment of the term, and results in the description given above. The other new point respecting the passing of the network of lumina into the single lumen at *g*, I saw something of before, although I had not fully understood it.<sup>2</sup> The vesicle and the duct from the vesicle to the exterior (*k*—*l*) need no further description. The lumen of this region is surrounded by numerous ciliated cells.

### Aulostoma.

The funnel is quite similar, and similarly placed, to the funnel in *Hirudo*; its existence has also been always heretofore denied. The structure of the remainder of the nephridium also closely resembles the structure found in *Hirudo*. It has been fully worked out by Schultze, and his results agree with my

<sup>1</sup> A. G. Bourne, 'Quart. Journ. Micr. Sci.,' vol. xxii, 1892, p. 337, "The 'Central Duct' of the Leech's Nephridium."

<sup>2</sup> A. G. Bourne, 'Quart. Journ. Micr. Sci.,' vol. xx, 1880, Pl. XXIV, fig. 2, *x*.

own. The chief point of difference from *Hirudo* is that in *Aulostoma* the ductules of the main lobe which collect at *e* (woodcut, fig. 7), pass at once into a single ductule which winds somewhat, but passes as a simple lumen perforating rounded cells (the "apical lobe") as far as *g*, where it changes the characters of its walls, the cells taking on the drain-pipe form.

### Hæmopis, Hæmadipsa.

I have discovered funnels in these genera similar in structure to the funnels in *Hirudo*. I may also state that the vesicle in *Hæmadipsa* is very large, and the apertures to the exterior, instead of being quite ventral in position, are actually dorsad of the edges of the flattened body.

### Nephelis and Trocheta.

The nephridium resembles that of other Gnathobdellidæ and presents only slight simplifications of structure. The chief difference lies in the structure and position of the funnel. I have obtained isolated preparations of the funnel in both genera, and so far as *Nephelis* is concerned I can testify to the correctness of Leydig's figure<sup>1</sup>, and *Trocheta* presents little variation upon that type.

There is following immediately upon the funnel a dilatation (not figured by Leydig) such as described above in *Pontobdella*, &c. This is seen in section in fig. 51, *r*; it contains the same mass of corpuscles as in the other genera. The funnel and its dilatation lie in the hollowing in botryoidal tissue described above as metacœlom (figs. 51 and 62, *neph. fun.*).

General Conclusions respecting the Nephridia.—The nephridia present a serial arrangement with regard to their metameric repetition. If my observations upon *Branchellion* should prove correct there is in this genus very little expression of such repetition; the tubules do, however, become more crowded together at regular intervals, no doubt an indication of segmentation. It must, however, be borne in mind that these observations rest upon the examination of a single young

<sup>1</sup> Leydig, "Bericht der König.-Zootom. Anst. zu Würzburg," Leipzig, 1849.

specimen, and they may be incomplete—a series of funnels or of external apertures, or both, may exist. *Pontobdella* presents a condition where the network remains connected throughout the body, the funnels and external apertures exhibiting a segmental arrangement. Woodcut, fig. 8, A, represents diagrammatically a portion of the nephridial network in

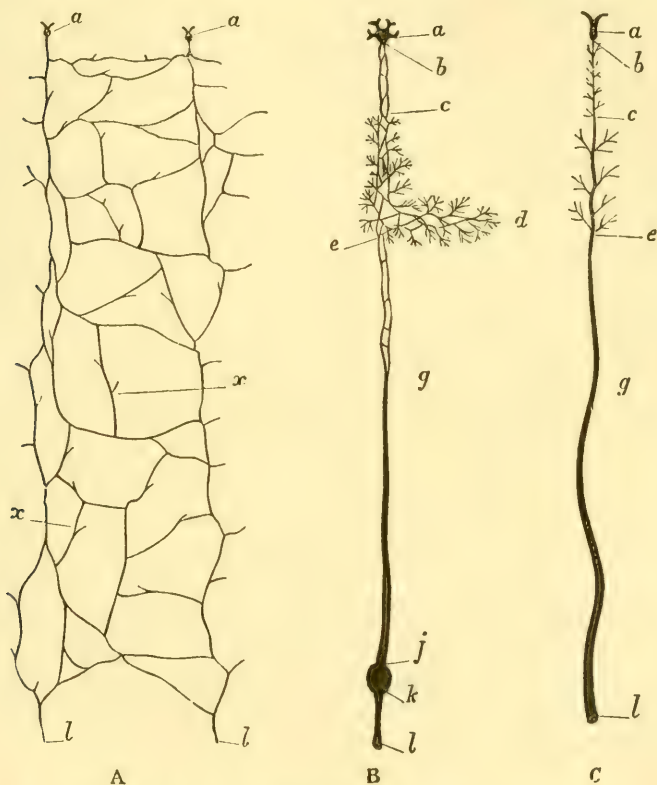


FIG. 8.—Diagrams of the nephridia in:—A. *Pontobdella* (two contiguous nephridia are shown). B. *Hirudo*. C. *Clepsine*. *a, a*. Funnels. *b—c*. Ducts in “testis-lobe.” *c—e*. Ducts in “main lobe.” *d*. In *Hirudo*, ducts in cæcal end of “main lobe.” *e—g*. In *Hirudo*, ducts in the “apical lobe.” *g*. The apex. *e—l*. In *Clepsine*; and *g—l*, in *Hirudo*, unbranched duct passing to the external aperture *l*. *j—k*. In *Hirudo*, the vesicle. *x, x*. Cæcal ductules in *Pontobdella*.

*Pontobdella* extending over two segments. The nephridium in *Hirudo* may be represented as in woodcut, fig. 8, B. This figure is to be compared with woodcut, fig. 4, the lettering of corresponding portions being the same. The special feature of the nephridium in *Aulostoma* and *Hirudo* appears to be the existence of the portion marked *d*, the cæcal end of the main lobe; and it seems possible that this is the remnant of a formerly existing connection between one nephridium and the next. The nephridium in *Clepsine* presents the simplest condition, and may be represented as in woodcut, fig. 8, c. The nephridium, then, in all cases, opens into cœlomic space on the one hand, and to the exterior on the other. Except, perhaps, in the region of the funnel, and from the vesicle to the exterior (the latter portion possibly an epiblastic invagination, cp. fig. 65), the lumen is contained in a perforated cell, and is continuous throughout. In some regions the cell so perforated contains a network of finer ductules which open within the cell into the duct—e. g. in *Clepsine* (woodcut, fig. 6, A B) and in *Hirudo* (fig. 7, A B, C D). In other regions the cell resembles a drain-pipe. At certain spots the duct in this latter condition re-enters a mass of cells as in *Hirudo* (woodcut, fig. 7, *d*), or even appears to re-enter a single cell, as in *Clepsine* (woodcut, fig. 6, *e*). With regard to this latter condition I may point out that numerous nuclei have been described<sup>1</sup> in cells so re-entered, and they may represent groups of cells fused together. It is a point needing further investigation. The condition of the nephridial network in *Pontobdella* and *Branchellion* is probably a very archaic one, and presents a distinct resemblance to that of the Planarians and Trematodes.

#### viii. ALIMENTARY CANAL.

I propose to say more about the alimentary canals upon a future occasion, and will simply refer now to Moquin Tandon's figures. A comparison of these shows that five regions may always be recognised, although they may differ very much in

<sup>1</sup> Lang, Schultze, loc. cit.



the extent to which they are developed, the amount of their branching, and, as I find, the histological character of their epithelium. In *Aulostoma* the alimentary epithelium is ciliated. This has not hitherto been observed, and is very interesting when we consider the difference in the nature of the food of this genus (earth-worms, smaller individuals of its own genus) and the food of *Hirudo*, &c. The regions are: 1, pharynx; 2, œsophagus and proventriculus; 3, digestive stomach; 4, intestine; and 5, rectum.

In *Hirudo* by far the largest portion is the œsophagus and proventriculus with its ten pairs of cæcal diverticula; this is followed by the digestive stomach, the very reduced portion with the so-called rectal cæca, and then the intestine and rectum. This very small stomach is related to the very slow absorption which takes place in *Hirudo*. In other genera the corresponding region is much more fully developed. There is probably no absorption taking place in the portion with the diverticula, the epithelium in this region possibly secreting a preservative or digestive juice. In transverse sections the cells may be seen giving off droplets of a mucous-like substance which have sunk into the blood-mass (fig. 66, *m*).

#### ix. PHYLOGENETIC CONCLUSIONS.

The place of the leeches in classification is a question which has at all times attracted a considerable amount of attention. I do not propose to give here any history of the older views upon the subject.

Two main views are held at the present day. Either the Hirudinea are Platyhelminthes specially related to the Trematodes and Triclada, or this resemblance is a superficial one, and they are more closely related to the Chætopoda.

The former of these views has been insisted upon by P. J. Van Beneden<sup>1</sup> and Leuckart,<sup>2</sup> who hold that they are allied to

<sup>1</sup> Van Beneden and Hesse, 'Récherches sur les Bdelloïdes ou Hirudinées et les Trématodes Marins,' Brussels, 1863.

<sup>2</sup> 'Die Mensch. Parasiten,' 1865.

the Trematoda. Carl Vogt<sup>1</sup> suggested, and Lang has insisted upon, their connection with the Triclada, and especially with *Gunda segmentata*. Professor Lankester refers them to the Platyhelminthes in his 'Notes on Embryology and Classification,' 1876.

On the other hand, Cuvier, Leydig, and de Quatrefages have dwelt upon the resemblances in their structure to Annelids, more especially as regards nervous and circulatory systems, and Huxley placed them with the Chætopods and Gephyræa in his group Annulata. Hatschek has insisted upon their close relationship with the Chætopoda. Balfour also removed them from among the Platyhelminthes, and treated them as an independent class allied to the Chætopoda.

In drawing phylogenetic conclusions with regard to any group of animals, we are led to consider certain series of facts—viz. (1) the amount of variability in any particular system of organs within the group itself; (2) the adult conditions of the systems of organs in the group in relation to that condition in allied groups; and (3) the ontogenetic history of the individual genera. I shall only consider here, as I have put forward no new developmental facts, the variability of structure in the group and the anatomical relations with other groups.

### 1.—Variability of Structure within the Group.

In few groups of the animal kingdom (in relation to their size) does the consideration of this question afford us more valuable results than in the Hirudinea. The importance of such considerations has been recently pointed out by Hubrecht<sup>2</sup> with respect to the nervous system of Nemerteans.

In the Hirudinea we have in this respect to note the striking correspondence—a correspondence evidently due to true homogeneity and not to mere homoplasy—in certain minute details, remarkable in themselves, between members of the group which, in other respects, present the widest divergence.

Let us consider, in the first place, the constancy of structure which occurs. The nervous system presents the greatest simi-

<sup>1</sup> Carl Vogt, 'Zoologische Briefe,' Frankfort, 1851.

<sup>2</sup> 'Quart. Journ. Micr. Sci.,' vol. xx, 1880, p. 276.

larity of structure throughout the group, and that structure is of a most highly developed nature. As in the higher Annelids and Arthropods there is a complete separation of ganglion cells from nerve-cord, the number of ganglia is the same in every genus, although this is not the primitive number, as is conclusively shown by the fact that certain ganglia fuse together during the development.

The posterior sucker exists and presents very minutely similar structure throughout the group.

The pharyngeal wall, whether so greatly modified as a whole as to be capable of withdrawal into the anterior region of the body, as in the Rhyncobdellidæ, or remaining as the anterior portion of the body, as in the Gnathobdellidæ, presents, as I have shown, a close agreement in its minute histological detail throughout the group.

The nephridial funnel, although differing entirely in position, while the nephridium itself presents the two extremes of variation in *Pontobdella* and *Clepsine*, shows an identity of essential structure in these two genera.

Although *Clepsine* differs as much as any leech from *Hirudo*, *Aulostoma* and *Nephelis* and the nephridium of *Clepsine* on the one hand and of these three genera on the other presents great differences in complication, yet the main duct has almost precisely the same course and re-enters (in itself a most specialised condition) the cellular masses or lobes through which it has already passed at precisely similar spots. At the same time the nephridial funnel of *Clepsine* as stated above resembles that of *Pontobdella* and differs widely from that of *Hirudo*; there is, moreover a well-developed vesicle in *Hirudo* and no trace of such in *Clepsine*.

Passing, in the second place, to consider specially the variability of structure which may occur we may note the following facts:

The anterior sucker, present in *Pontobdella*, *Piscicola*, and *Branchellion* is as little represented in their ally *Clepsine* as it is in the members of the *Gnathobdellidæ*.

The number of annuli which build up a somite, although

very constant within a genus, differs in different genera. The amount of cœlom present, the amount of true vascular system developed, differs very greatly among the genera inter se. The funnel of the nephridium may open into very different portions of the system of cœlomic spaces.

The alimentary canal always presenting the same regions, may differ entirely in the presence or absence of cæcal diverticula in those regions in either group (cp. *Pontobdella* with *Clepsine*, *Trocheta* with *Hirudo*).

The two genera (*Clepsine* and *Pontobdella*) most closely allied in most respects present the widest difference of structure in their nephridia.

Lastly, although the groups of the *Rhyncobdellidæ* and *Gnathobdellidæ* are so well separated by the characters of the pharynx, the presence or absence of hæmoglobin in the blood, the vascularisation of the pigmented connective tissue, and the difference in the manner of communication between sinus and vessel, yet we may have such close correspondence between a single genus of the one group and the genera of the other group as that mentioned above as occurring in the arrangement of the main duct of the nephridium in *Clepsine* on the one hand and the *Gnathobdellidæ* on the other.

I may repeat that it is not the variability merely to which I am drawing attention but the curious distribution in its amount, with regard to systems of organs or portions of such, among the various genera.

What conclusions are to be drawn from these facts?

They point to the persistence of the genera of Leeches existing at the present day over a very long period; in other words, they demonstrate the very archaic nature of the group. In the existing genera there is no series of modifications, it is not possible as it is in so many groups to point to a genus or genera as being more archaic than the rest, these latter exhibiting serial modifications with either a progressive or a retrograde tendency. We find in one genus what is apparently an archaic condition of a certain system of organs, but then we have to look in a widely removed genus for the archaic condition



of another system; while certain systems, the nervous for instance, present a most marked uniformity throughout the group and that in no primitive condition. It is not unusual to find a nervous system in a primitive condition in animals otherwise highly developed, e. g. *Peripatus*, but it is unusual to find an organ so primitive as the nephridial tubules of *Pontobdella* appear to be, in connection with such a well-developed nervous system. This becomes more noteworthy when we find that in an allied genus *Clepsine* presenting a precisely similar nervous system, a highly developed condition of the nephridia obtains. We must regard the Hirudinea as presenting a number of isolated genera belonging to two groups, a large number of intermediate forms having been lost. These genera appear to have had an ancestor presenting as high a development of each system of organs as is found in any single genus of living Hirudinea.

## 2.—Anatomical Structure in relation to Allied Genera.

There can be no doubt that we must look for structural resemblances to the Leeches in the Platyhelminthes on the one hand, and in the Chætopods on the other: *Gunda segmentata* among the Platyhelminthes, and *Branchiobdella* among the Chætopoda, presenting special resemblances to the group. But I consider it quite impossible to show that the Leeches are either more highly developed Triclada or that they are degenerate Chætopoda; in other words, the genetic relations are indirect, and not direct, as has been sometimes stated.

Although in most respects the Leeches present a much more highly developed condition than is found in any Platyhelminth, they do exhibit certain well-marked affinities with the latter, in both positive and negative characters. The presence of median generative pores in similar positions in all the genera of Leeches, in connection with a hermaphrodite condition, is a very marked Platyhelminth character.

The suckers also resemble those of Trematodes, but, as

Hatschek<sup>1</sup> has pointed out, the posterior sucker is composed of a certain number of fused segments. The segmentation here is, however, only part of the segmentation which has gradually asserted itself in the course of the ancestral development of the Leeches.

The general arrangement of muscles corresponds with that obtaining in the Triclada. The oblique muscular layers in the body-wall and the dorso-ventral muscles are unknown in any Chætopod.

The structure of the pharynx in the Rhynchobdellidæ, as Lang has pointed out, agrees very minutely with that in *Gunda segmentata*.

The Leeches differ markedly from the Chætopoda in the absence of parapodia and of setæ, but it must be remembered that *Polygordius* is devoid of setæ, and *Branchiobdella* also has no setæ.

The Leeches agree with some Chætopods in the presence of clitellum and in the practice of forming cocoons. The branchial apparatus resembling that of some Chætopods, though present only in a well-developed condition in *Branchellion*, is represented, as I have shown, in all the other genera of Rhynchobdellidæ.

The segmented condition of the Leeches stands upon rather a different footing. Animals presenting a metameric segmentation are not necessarily allied; it is quite certain that such segmentation has arisen at different times in different groups of the animal kingdom, and it is quite possible that the segmentation of a Leech has come about by a process of dysmerogenesis, as opposed to that of a Chætopod, which has been attained by a synthesis of a eumeristic colony—a process of eumerogenesis.<sup>2</sup>

With regard to what represents cœlom in the Leeches, it seems certain that this has once been more fully developed; the existence of remnants of cœlomic epithelium, of a highly

<sup>1</sup> 'Arb. aus dem Zool. Inst.,' Wien and Triest, tom. 1, 1878, p. 340.

<sup>2</sup> Cp. E. Ray Lankester, 'Encyclopædia Brit.,' 9th edition, art. "Hydrozoa—Hypothesis of the Individuation of Organs."

organised nerve-cord lying in a much less highly organised cœlom, and of well-developed funnels to the nephridia, all point to this. The Leeches have thus had an ancestor which in possessing a cœlom was already a great advance upon any Platyhelminth form—if we accept, as I cannot yet do, Lang's views of the cœlenterate character of Platyhelminths.

Lang<sup>1</sup> has put forward the view that the paired diverticula of the alimentary canal in Leeches are homologous with the cœlomic diverticula of the ancestral alimentary canal of the Enterocœla, and thus compares them with the diverticula of the alimentary canal of the Triclada; but as it is probable, that in Leeches the cœlom is represented by a very different system of spaces, such diverticula cannot have the special connection with the diverticula in the Triclada which Lang suggests, unless the latter too do not really represent cœlomic diverticula,—a representation which Lang has suggested rather than proved.

The consideration of the anatomical facts now ascertained with regard to the adult forms of the various genera of Leeches appears to me, in the absence of more definite embryological information than we possess, to lead us to no very certain conclusion as to the affinities of this group. Everything in this question depends on a perfectly reliable knowledge of the embryological history, in the different genera of Leeches, of the spaces which I have treated of in this memoir under the name "cœlom," and a simultaneous knowledge (more complete than that which we owe to Lang) of the embryological history of the intestinal cœca and nephridia of Planarians and Trematodes, and of the presence or absence of a temporary "cœlom" in the embryonic condition of the latter.

Assuming for the moment that Lang is right as to the definite cœlenterate structure of the Planarians and Trematodes, and that such structure is not a degeneration but a primary character, then it would be necessary to place the Leeches in a distinct group, characterised by its "cœlom" into which the large nephridial funnels open, by its vascular system,

<sup>1</sup> 'Mitthl. Zool. Stat. Neapel,' Bd. 3, p. 233.

and by the strongly-marked metameric segmentation of its nerve-cord and some other organ-systems. But these characters, although they are shared by the Leeches with the Chætopoda, do not appear to me to be of value as indications of affinity. They are merely the expressions of a necessary method of elaboration of structure which probably occurs in several parallel groups. A cœlom and a segmentation of the body are not specific indications of community of ancestry, and cannot be recognised in a phylogenetic classification by associating the Leeches in the same group with the Chætopoda.

On the other hand such minute details as the structure of the muscles of the body-wall, the structure of the pharynx, the median position of the male and female genital pores united in one individual, and the general features of the structure of the reproductive organs—common to the Leeches and the Triclada—cannot, I venture to think, be regarded with any probability as owing their agreement in the two groups to mechanical necessity apart from heredity. They imply the existence of an ancestor common to the Leeches and the Triclada (with which of course go also the Trematodes and the Cestodes) possessing these characters. Whether the Leeches have advanced very greatly beyond the degree of elaboration of organisation exhibited by that common ancestor, or whether the ancestor was near in all points except segmentation to the existing Leeches, whilst the Triclada and Trematodes have degenerated, is a matter for further inquiry.

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## EXPLANATION OF PLATES XXIV—XXXIV,

Illustrating Mr. A. G. Bourne's memoir "On the Hirudinea."

*General References.*

*cl.* Clitellum. *n. c.* Nerve-cord. *gn.* Ganglion. *g. p.* Genital pore. *neph. ap.* Nephridial external aperture. *cu.* Cuticle. *ep.* Epidermis. *der.* Dermis. *al. ep.* Alimentary epithelium. *gl.* Gland-cell. *gl. m.* Mucous gland. *gl. s.* Salivary gland. *gl. s. d.* Ducts of salivary glands. *gl. cl.* Clitellar gland. *gl. cl. d.* Ducts of clitellar glands. *gl. pr.* Prostomial gland. *c. t.* Connective tissue. *ma.* Matrix of connective jelly. *a.* Indifferent connective-tissue corpuscles. *b.* Vacuolated cells, fat cells. *c.* Cells forming fibres. *d, e, f.* Non-vascular pigmented cells. *g.* Cells forming botryoidal or vaso-fibrous tissue. *v.* Vessel. *s.* Sinus. *d. v.* Dorsal vessel. *d. s.* Dorsal sinus. *v. v.* Ventral vessel. *v. s.* Ventral sinus. *l. v.* Lateral vessel. *l. s.* Lateral sinus. *Cæl.* Cælotomic space. *Cæl. ep.* Cælotomic epithelium. *cap.* Capillary vessel. *m. rad.* Dorso-ventral or radial muscles. *m. circ.* Circular body muscles. *m. obl.* Oblique body muscles. *m. long.* Longitudinal body muscles. *m. cut.* Cutaneous muscles. *m. al. c.* Circular muscles of the alimentary canal. *m. al. l.* Longitudinal muscles of the alimentary canal.

FIG. 1.—*Pontobdella muricata*, dorsal view.

FIG. 2.—Similar view in outline. Somites numbered 1—23.—Annuli of a single somite, i—iv. *a.* Anterior sucker. *b.* Posterior sucker. *an.* Anus. *c.* Clitellum.

FIG. 3.—*Pontobdella muricata*, opened along the median dorsal line, slightly diagrammatic. *c. g.* Supra-œsophageal ganglion. *g.* 1—23, the post-oral ganglia. *œ.* Passage for the œsophagus piercing the ganglionic mass. *n. f.* Nephridial funnels. *n. a.* Nephridial apertures to exterior. *n.* The nephridial network. *te.* 1—6, the testes. *pr.* The so-called prostatic gland. *ov.* The ovaries. *g. p. ♂* and *g. p. ♀*. Male and female generative pores.

FIG. 4.—*Hirudo medicinalis*, ventral view. Somites numbered 1—23.—Annuli of a single somite, i—v. *a.* Mouth, anterior sucker. *b.* Posterior sucker. *c.* Clitellum. *g. p. ♂*. Male genital pore. *g. p. ♀*. Female genital pore. *n. a.* Nephridial apertures to the exterior.

FIG. 5.—*Hirudo medicinalis*, opened along the median dorsal line, slightly diagrammatic. *cer. g.* Supra-œsophageal ganglion. *g.* 1—23, the post-oral ganglia. *œ.* Passage for the œsophagus piercing the ganglionic mass. *l. v.* Lateral vessel. *l. l.* Latero-lateral branch. *l. ab.* Latero-abdominal branch. *l. d.* Latero-dorsal branch. *neph.* 1—17, the seventeen pairs of

nephridia. *te.* 1—9, the nine pairs of testes. *ep.* Epididymes. *pe.* Penis. *ov.* The ovisacs. *gl.* Glandular enlargement of the oviducts.

FIG. 6.—*Pontobdella muricata*, portion of external surface. *aa.* The median dorsal line. *bb.* The median ventral line. I, II, III, and IV, the annuli of one somite. *Ia.* The most anterior annulus of the next somite. *neph.* External apertures of the nephridia. (N.B.—These are wrongly placed in *Ia*: they should have the same position as in I.)

FIG. 7.—*Pontobdella muricata*, similar view. Clitellar region.—5. The only annulus of somite 5. 6. The anterior clitellar somite. *g. p. ♂.* The male genital pore. 7. The posterior clitellar somite. *g. p. ♀.* The female genital pore. 8. Anterior annulus of somite 8. *a, b.* Rings devoid of papillæ at either end of the clitellum.

FIG. 8.—*Pontobdella muricata*, diagram of an opened somite from the central region of the body, showing the relation of the internal organs to the annuli. On the right side is shown only the nephridial network. *neph. fun.* Nephridial funnel. *neph. ap.* Position of the external aperture. *n.* and *g. n.* Nerve-cords and ganglia. *v. v.* The ventral vessel, lying within *v. s.* the ventral sinus. *d. v. s.* Dorso-ventral sinus, containing branch of nerve-cord and nephridial funnel. *l. v.* Lateral vessel. *l. s.* Lateral sinus (diagrammatic). *br.* Branchial dilatation. *br. s.* Sinus connecting it with lateral sinus. *br. v.* Vessel opening into it. *x.* Muscular bands in the walls of lateral sinus. *m. rad.* Dorso-ventral muscles. *t.* Position of the testis. The numbers I, II, III, IV, and *Ia*, mark the annuli as above.

FIGS. 9—14 represent portions of transverse sections, the relative size of the various histological elements is accurately represented. For explanation of the reference letters, see General References.

Fig. 9.—*Pontobdella*.

Fig. 10.—*Clepsine*.

Fig. 11.—*Piscicola*.

Fig. 12.—*Branchellion*.

Fig. 13.—*Hirudo*. } *k* marks the point of transition of botryoidal

Fig. 14.—*Trocheta*. } into vaso-fibrous tissue.

FIG. 15.—*Hirudo*.—Portion of a section stained with gold, showing sensory epidermic cells, *ep. sens.*, and nerves, *n.*

FIGS. 16—24.—From transverse sections through the anterior region of the body. See General References. Figs. 16, 17, 19, 21, and 23 are drawn to the same scale; Figs. 18, 20, 22, and 24 are more highly magnified.

Fig. 16.—*Trocheta*. *te.* Loops of testicular tubules, which are carried forward into this region.

Figs. 17 and 18.—*Branchellion*.

Figs. 19 and 20.—*Piscicola*.

Figs. 21 and 22.—*Clepsine*.

Figs. 23 and 24.—*Pontobdella*.

FIG. 25.—*Pontobdella*. Connective jelly, with corpuscles. *a*. Elongated corpuscle forming a fibre; nucleus in some dividing. *b*. Similar corpuscle, but branched. *c*. Vacuolated corpuscle.

FIG. 26.—*Pontobdella*. Corpuscle containing refringent granules of various sizes, *gr.* *n*. Nucleus.

FIG. 27.—*Piscicola*. "Fat cells." *end*. Endoplastic globules. *n*. Nucleus.

FIG. 28.—*Aulostoma*. *a*. Unmodified connective-tissue corpuscles. *b*. Similar corpuscles, dividing and elongating. *d*. The corpuscles, as a result of division, forming rows, and at *e*. breaking down to form a lumen, thus ultimately giving rise to botryoidal tissue *f*.

FIG. 29.—*Pontobdella*. Very young embryonic corpuscles, elongating and developing pigment.

FIG. 30.—*Pontobdella*. Elastic fibres, fresh, teased.

FIG. 31.—*Piscicola*. Corpuscles branching and developing pigment, taken from a deep layer.

FIG. 32.—*Piscicola*. Similar corpuscles, taken from a more superficial layer. They are more branched, and there are fewer nuclei.

FIG. 33.—*Hæmadipsa ceylonica*. Mass of branched corpuscles which have fused together. From a superficial layer.

FIG. 34.—*Branchellion*. Similar mass from a superficial layer.

FIG. 35.—*Pontobdella*. Corpuscles which have developed pigment but retained their shape. Deep layers.

FIG. 36.—*Pontobdella*. Similar corpuscles, showing their arrangement in rows.

FIG. 37.—*Pontobdella*. Similar cells, which have undergone further division. Median layers.

FIG. 38.—*Pontobdella*. Similar cells, which have become much branched. Superficial layers.

(FIGS. 39—43 occur on Plate XXXIII.)

FIG. 39.—*Pontobdella*. Blood-corpuscles, fresh. *n*. Nucleus.

FIG. 40A.—*Nephelis*. Blood-corpuscles, fresh. *n*. Nucleus.

FIG. 40B.—*Hirudo*. Blood-corpuscles, fresh. *n*. Nucleus.

FIG. 40C.—*Ditto*. *Ditto*, weak acetic acid. *n*. Nucleus.

FIG. 40D.—*Ditto*. *Ditto*, magenta. *n*. Nucleus.

FIG. 41.—*Pontobdella*. Cœlomic epithelium cells from ventral sinus. *a*. Connective-tissue layer.

FIG. 42.—*Pontobdella*. Similar cells. This is a very complete instance of their occurrence from a transverse section of a branch of the ventral sinus. *l. n*. The lateral nerve branch, about to divide into a dorsal and ventral branch. *cœl*. Cœlomic space (sinus). *a*. Connective tissue layer.

FIG. 43.—*Pontobdella*. Similar cells, seen in a flat section lining the

wall of a sinus. One cell may be seen recently divided. *a.* Connective-tissue layer.

(FIGS. 44—47 occur on Plate XXXIV.)

Fig. 44.—Pontobdella. A "lymphatic nodule." *m. n.* Blood-vessels. *o.* The connective-tissue capsule. *p.* The trabecular network. *q.* Enclosed amœboid corpuscles. The majority of these corpuscles have been omitted to show the trabecular network.

Fig. 45.—Pontobdella. Portion of the dorsal sinus and vessel. *d. s.* Dorsal sinus. *m.* Muscular band in its wall. *d. v.* Dorsal vessel lying in the sinus. The scattered nuclei in the walls are shown.

Fig. 46.—Pontobdella. Portion of the lateral sinus and contained vessel. *l. s.* Lateral sinus, with an almost obliterated lumen. *m.* Muscular band in its wall. *l. v.* Lateral vessel with its densely-muscular walls.

Fig. 47.—Pontobdella. *a.* Capillary vessel. *c.* The cuticle of the walls. *p.* The protoplasmic lining, with nuclei *b.* *a.* Amœboid corpuscles running here and there into plasmodial masses.

Fig. 48.—Hirudo. Cells from the apical lobe of the nephridium. *d.* Duct. *a.* Nuclei in its walls. Perforated cells, *c. c.*, are indicated in the drawing on each side of this main duct. *b. b.* Their nuclei. *duct.* Their ductules. *cap.* Blood capillaries.

FIGS. 49—58 occur on Plate XXXI.

Fig. 49.—Hirudo. "Funnel" of the nephridium. *a.* The many-lobed mass surrounding the dilated portion and constituting the "funnel." *b.* The extremity of the "testis" lobe.

FIG. 50 A, B, C.—Hirudo. Various ciliated cells from the surface of the funnel. These do not exhibit any actual apertures, but are of a spongy nature, and in some cases present an intracellular lumen, *l.* *n.* Nuclei. *m.* Connective tissue upon which they lie.

FIG. 51.—Trocheta. Botryoidal sinus containing the funnel of the nephridium, seen in transverse section. *c. t.* Connective tissue forming a capsule. *m.* Small muscular fibres developed in the walls. *ep.* Cells of botryoidal tissue forming an epithelium. *t.* Communication with the blood system. *neph.* Nephridial cell. *r.* Diverticulum following on the mouth of the funnel, *f.*, containing degenerating blood-corpuscles. *cœl.* Cœlomic space. The actual communication between the nephridial cell and the dilated neck of the funnel is not shown.

FIG. 52.—Clepsine. Ventral sinus with nephridial funnels, from transverse sections. Diagrammatic. *v. s.* Ventral sinus. *v. v.* Ventral vessel. *n. g.* Nerve-ganglion, giving off lateral branches. *f. f.* Funnels of the nephridia. *v.* Diverticulum following upon the mouth of the funnel, filled as in Fig. 51. *neph.* Nephridial cells.

FIGS. 53 and 54.—Pontobdella. Funnel and diverticulum of the nephri-



dium lying in the perinephrostomial sinus, *s.* *f.* The funnel; with *n.n.* nuclei, and *v.* vacuoles. *a.* The neck of the funnel, with ciliated lumen and nucleus. *r.* The diverticulum, containing filaments and masses of blood-corpuscles. A very thin layer of cells may be seen covering the neck of the funnel and parts of the diverticulum. Cœlomic epithelium, *ep.* In Fig. 53 several cœlomic epithelium cells, *cœl.ep.*, are seen floating in the sinus. *b.* Blood sinus, communicating with the perinephrostomial sinus; the other communicating sinus is not visible. *x.* Point of communication of the nephridial tubules with the funnel.

FIG. 55.—Pontobdella. Filaments contained in the diverticulum of a nephridial funnel, seen after staining with iodine.

FIG. 56.—Pontobdella. Surface view of portion of the nephridial tubules, to show the markings upon the surface.

FIG. 57.—Pontobdella. Portion of the nephridial tubules, showing nodules on the surface. *n, n.* Nuclei.

FIG. 58.—Pontobdella. Portion of the nephridial tubules, showing blind endings. *n.* Nucleus.

FIG. 59 (Plate XXXII).—Pontobdella. (Nitric-acid, glycerine preparation.) *neph.* Network of nephridial tubules, with *n. n.* nuclei. *cap.* Network of capillaries. *gl. cl.* Clitellar gland-cell and ducts. *b, b.* Connective-tissue corpuscles.

FIG. 60 (Plate XXXII).—Pontobdella. Network of nephridial tubules in the fresh state, drawn to the same scale as Fig. 59, showing the extent to which the lumen becomes distended. *l.* Lumen. *n. n.* Nuclei.

FIG. 61 (Plate XXXIII).—Hirudo. Transverse section passing through a pair of testes and nephridial funnels, but not a nephridium. *al.* The main portion of the alimentary canal cut through in the centre, with sections of cœcal diverticula on either side. The pigment in the body wall is not shown, a small portion being put in at *x.* See general references.

FIG. 62 (Plate XXXIII).—Nephelis. Transverse section passing through nephridial funnels and testes. See general references.

FIG. 63 (Plate XXXIV).—Pontobdella. Halves of two transverse sections taken from different regions of the body. The left-hand half passes through the first annulus of a somite, showing the papillæ in section, one of these containing the excretory canal of the nephridia, *neph. apt.*, and another the branchial dilatation, with portions of the sinus coming to it, *br. s.* It also passes through a nerve-ganglion, *n. g.* The right-hand half passes between two annuli, and this shows little dermic region. It passes through a testis, *t.*

FIG. 64 (Plate XXXIV).—Clepsine. Transverse section passing through two of the diverticula of the alimentary canal, and also through a pair of nephridial funnels, *neph. fun.*

These four figs., 61—64, are drawn to scale. For reference letters, see above—general references.

FIG. 65 (Plate XXXII).—*Pontobdella*. Portion of a transverse section passing through the excretory portion of the nephridium. *neph. apert.* The aperture to the exterior, lined by *cu.* a cuticle. At *x*, the nephridial tubules open into this invaginated portion. *neph.* Nephridial tubules.

FIG. 66 (Plate XXXII).—*Hirudo*. Drawn from a transverse section by Mr. W. E. Roth, B.A., of Magdalen College, Oxford. *d. v.* Dorsal vessel, containing shrunken blood-clot. *g.* Botryoidal tissue. *al. ep.* Alimentary epithelium. *m.* Mucous droplets passing into *fd.*, the food-blood in the alimentary canal.

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## On the Nervous System of *Antedon rosaceus*.

By

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With Plate XXXV.

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DURING a recent visit to the Zoological Station, at Naples, I devoted some time to an investigation of the nervous system of *Antedon*, with the object of testing by actual experiment the validity of the rival doctrines which have been advanced concerning it of late years.

I propose in the present paper to give (1) a brief sketch of the general organisation of *Antedon*, in order to define the terms employed, and to make the following descriptions more readily intelligible; (2) a short historical account of the controversy regarding the nervous system of *Antedon*, including the present position of the question; (3) an account of my own experiments and observations; and (4) a discussion of certain points of morphological interest affected by the conclusions arrived at in the preceding section.

### I. GENERAL DESCRIPTION OF ANTEDON.

*Antedon*<sup>1</sup> consists of a central disc from which radiate five pairs of long arms, fringed with pinnules.

<sup>1</sup> For fuller descriptions, *vide* Carpenter, "Researches on the Structure, Physiology, and Development of *Antedon rosaceus*," part i, 'Phil. Trans.,' 1866; and Ludwig, 'Morphologische Studien an Echinodermen,' Bd. i, Abth. i;

The disc consists of a calcareous cup or calyx (vide fig. 1), and of the visceral mass which is lodged within the cavity of the calyx, and contains the whole of the alimentary canal and important parts of the vascular, sensory, and other systems.

The surface of the visceral mass covered by the calyx is commonly called the dorsal or aboral, the opposite one being the ventral or oral surface. In, or near, the middle of the latter is the mouth (fig. 1, *r*); this leads into a convoluted alimentary canal (*s*) which ends in an anus placed at the top of a conical chimney-like projection, which arises from the oral surface of the disc not far from its edge and interradially, i. e. between two pairs of arms.

To the dorsal surface of the calyx are attached from twenty to thirty jointed filaments or cirri (fig. 1, *p*) by which the animal attaches itself to foreign bodies. The calyx itself consists of a number of calcareous plates arranged as follows (cf. figs. 1 and 3):—In the centre is a single pentagonal centrodorsal plate (C. D.), to the dorsal surface of which the cirri are attached, while the ventral surface is hollowed out in its centre to form a cup-shaped cavity closed above by a thin calcareous plate—the Rosette (R.); more peripherally the centrodorsal plate supports a ring of five plates called First Radials (R.<sub>1</sub>). To the outer surfaces of these are connected five Second Radials (R.<sub>2</sub>) which overlap and almost entirely conceal the First Radials from the dorsal surface (fig. 1), and beyond the second comes a set of five Third Radials (R.<sub>3</sub>).

Each Third Radial bears distally a pair of First Brachials (figs. 1 and 3, Br.), which are the first of a series of short calcareous joints placed end to end and extending the whole length of the arms.

The spaces between the radials and between the basal joints of the arms as far as the fourth brachials are filled up by uncalcified portions of the perisome or body wall which thus complete the calyx.

and for a very excellent summary of recent researches, vide P. H. Carpenter, "The Minute Anatomy of the Brachiote Echinoderms," this Journal, vol. xxi.



The several joints of the arms are moveable on one another. Movement towards the oral or ventral surface, which will be called flexion, is effected by muscles (figs. 1 and 2, *u*) running between the successive segments; extension or movement towards the dorsal surface is on the other hand almost entirely due to the action of elastic ligaments placed nearer the dorsal surfaces of the segments.

The dorsal and lateral surfaces of the arms are covered by an extremely thin layer of integument, but along the ventral surface the soft parts are much thicker and exhibit a considerable complexity of structure. Running along the ventral surface of each arm is a longitudinal furrow, the ventral or ambulacral groove (fig. 2, *i*), bordered on either side by a fold of perisome, the edge of which is notched into a series of concentric leaflets at the base of each of which is a group of three hollow tentacles (*k*).

The ambulacral groove is lined by a special ambulacral epithelium which is columnar and ciliated and much thicker than the non-ciliated epithelium covering the rest of the body. Beneath the columnar cells is a fibrillar layer (fig. 2, *h*) spoken of as the subepithelial band. This consists of very slender fibrils arranged for the most part longitudinally, and so appearing as fine dots in transverse sections of the arm; interspersed among the fibrils are very small nucleated cells. The subepithelial band in *Antedon rosaceus* is continuous with the ambulacral epithelium, of which it may be described as forming the deepest layer; it is traversed vertically by strands which are continuous on the one hand with certain cells of the columnar epithelium, and on the other with a connective-tissue stratum underlying the band. In other species the subepithelial band appears from the descriptions of Ludwig and others to be separated from the ambulacral epithelium by a very thin connective-tissue lamella.

At the bases of the arms the ambulacral grooves are continued on to the disc; those of each pair of arms unite together and so give rise to five radial grooves which run over the surface of the disc to the mouth, where they meet. Round the mouth

the subepithelial bands of the five radial grooves unite to form a pentagonal ring.

The tentacles, as described above, are hollow; their cavities communicate with a longitudinal canal (fig. 2, *l*) which runs along the arm just below the subepithelial band. These radial ambulacral canals are continued into the disc and open into a circular canal round the mouth (fig. 1) from which short branching canals are given off ending in open mouths communicating with the body cavity.

Besides the radial ambulacral canal, each arm contains also three diverticula of the body cavity or cœlom. Of these the most ventrally situated (fig. 2, *m*) is called the subtentacular canal and is commonly divided as in the figure by a median vertical partition; the most dorsally placed canal (fig. 2, *n*) is called the cœliac and communicates at the end of the arm with the subtentacular. The third or genital canal is placed between the other two and lodges the cord-like genital gland; it is very small in the arm, but much larger in the pinnules.

In the centre of the visceral mass is a plexiform structure (fig. 1, *g*) the real nature of which has been much disputed, but which, according to Ludwig and P. H. Carpenter, is part of the vascular system from which branches are given to all parts of the body, and among others a radial ventral vessel down each arm in the substance of the subepithelial band. This central plexus passes down through the central canal formed by the First Radials, passes through a hole in the middle of the rosette, and enters the cavity in the centrodorsal plate, where it expands to form a sac divided by vertical septa into five radial compartments, and hence called the chambered organ (fig. 1, *f*).

The chambered organ is surrounded by a thick fibrillar investment (*d*), known as the central capsule, and this is in connection with a system of fibrillar bands which run down the arms in the substance of the calcareous joints, and are hence called axial cords (figs. 1, 2, 3 *a*).

The connection between the central capsule and the axial

cords is rather complicated; but it is necessary to describe it in some detail, as it is with these parts that we shall be specially concerned later on.

The central capsule is lodged as we have seen in the hollow of the centrodorsal plate and is covered on its ventral surface by the rosette; it forms a complete investment to the chambered organ (fig. 1) excepting where it is perforated by the central plexus in the middle of the ventral surface. The dorsal and lateral walls are, as shown in the figure, thicker than the ventral.

From the dorsal surface are given off processes to the cirri (fig. 1, *e*) each of which is traversed down its centre by a vessel derived from the central plexus.

From the margin of the central capsule arise five short inter-radial processes (figs. 1 and 3), which passing ventralwards and slightly outwards bifurcate into right and left branches between the centrodorsal plate and the First Radials. These branches diverging from one another enter the substance of the First Radials, and then unite in pairs, the right branch of one interradial stem uniting with the left branch of the one next to it, to form five stout radial nerves (fig. 3) which run outwards in the substance of the First and Second Radials. On reaching the boundary line between the Second and Third Radials each of these radial cords divides into two branches right and left, which, traversing the Third Radial, enter the right and left arms respectively of the pair, along which they pass as the axial cords (figs. 1, 2, 3 *a*), in the substance of the brachials or calcareous segments of the arms.

Besides the connections described above there are certain others which must be noticed. A pentagonal commissure (fig. 3) connects all the branches together immediately after they have entered the First Radial. There is also a further connection in each Third Radial between the branches into which the radial cord divides to supply the two arms of the pair; this connection, as shown in fig. 3, consists of a transverse commissural band of fibres, and a chiasma formed by two obliquely placed bands which cross one another and furnish

additional communications between the right and left axial cords.

In the arms the axial cords lie in tubular channels perforating the calcareous joints (figs. 1 and 2). Each cord gives off alternately right and left stout branches, which enter the pinnules (fig. 2, *c*), in which their relations are the same as in the arms themselves. Besides these, finer branches are given off, both from the axial cords themselves and from the pinnule branches, which, passing through the calcareous joints, can be traced into very intimate relation with the muscles moving the arm-joints on one another, with the tentacles and the crescentic leaflets bordering the ambulacral groove, and with the tegumentary covering of the arms generally.

Histologically, the central capsule and the various cords in connection with it consist principally of very delicate fibrils, arranged for the most part longitudinally, and having interspersed among them very small nucleate cells, both the cells and fibrils closely resembling those of the subepithelial bands of the ambulacral grooves. Other fibres, of more irregular size and distribution, which traverse both the capsule and cords in various directions, appear to be of the nature of connective tissue, and to correspond to the vertical strands in the subepithelial bands. Externally both the capsule and the cords are invested by a layer of cells, which are much larger than the small ones found in the substance of the cords, stain deeply, and give off branching processes which are in very close relation with the reticulum forming the organic basis of the skeletal parts. This external layer of cells appear to me to be a mere investment of the cords and to be no part of their real substance.

The pinnules of each arm arise alternately from the right and left sides, each of the brachials except the first bearing one pinnule. The structure of the pinnules is, with certain exceptions, the same as that of the arms, each having an ambulacral groove, subepithelial band, tentacles, ambulacral, subtentacular, genital and coeliac canals, a branch of the axial cord, &c.; the genital rachis, however, which is only a slender



cord in the arms, dilates in the pinnules to form the genital glands. The proximal or oral pinnules, i. e. those borne by the Second Brachials, differ markedly from the others; they are longer than the rest, and habitually bend inwards, so as to arch over and cover the disc; they have no tentacles<sup>1</sup> and no ambulacral grooves, the ciliated epithelium of the grooves and the subepithelial bands being both absent;<sup>2</sup> they possess, however, like the other pinnules, branches of the axial cords of the arms.

## II. HISTORICAL SKETCH.

I propose, in this section, to notice briefly the principal views that have been advanced concerning the nervous system of Antedon.

Müller,<sup>3</sup> in 1841, gave the first account of the genital rachis in the arms of Antedon, but mistook it for the nervous system, and described it as such; he also mentioned the axial cords, but described them as vessels.

In 1865 Dr. Carpenter, in his 'Memoir on the Structure, Physiology, and Development of *Antedon rosaceus*,' corrected Müller's mistake concerning the genital rachis; and, with regard to the axial cords, stated<sup>4</sup>: "It will be shown, in the second part of this memoir . . . that a system of branching fibres proceeding from the solid cord that traverses the axial canal of each calcareous segment of the rays and arms is traceable on the extremities of the muscular bundles, and reasons will be given for regarding these fibres as probably having the function of nerves, though not exhibiting their characteristic structure."

In 1872 Baudelot<sup>5</sup> called attention to the anatomical and

<sup>1</sup> Carpenter, 'Phil. Trans.,' 1866, p. 702.

<sup>2</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," part ii, 'Journal of Anatomy and Physiology,' vol. xi, p. 90.

<sup>3</sup> J. Müller, "Ueber den Bau des *Pentacrinus Caput Medusæ*." *Physikalische Abhandlungen der Königlichen Akademie des Wissenschaften zu Berlin*.

<sup>4</sup> Carpenter, 'Phil. Trans.,' vol. 156, 1866, p. 705.

<sup>5</sup> Baudelot, "Études Générales sur le Système Nerveux," 'Archives de Zoologie Expérimentale,' tome i, 1872, p. 211.

histological resemblances between the axial cords of *Antedon* and the radial nerve-cords of other Echinoderms. He mentioned the pentagonal commissure in the calyx and the branches of the axial cords to the pinnules, and described the cords as consisting of fibrils cemented together by a finely granular substance and with very small cells imbedded in the fibrillar tissue. He appears to have been unacquainted with Dr. Carpenter's work, and, in spite of the resemblances he points out so clearly, denies absolutely the nervous nature of the axial cords without stating definitely his reasons for so doing.

Semper, in 1874<sup>1</sup>, published an independent refutation of Müller's error as to the genital rachis, and suggests concerning the nervous system, in ignorance of Dr. Carpenter's statement quoted above, "It might even be possible that the cord in the interior of the calcareous skeleton (i. e. the axial cord) is a nervous cord; and if so, then the so-called heart situated in the calyx would certainly have to be looked upon as a ganglion." Semper also suggests that a fibrous cord described by Perrier<sup>2</sup> as lying above, i. e. on the ventral side of the tentacular canal, may also belong to the nervous system. He confirms the existence of this cord, and refers to it as  $x$  in a diagrammatic transverse section of an arm.

In an addendum to the translation of Semper's paper<sup>3</sup> and in a second communication to the Royal Society on the structure, physiology, and development of *Antedon rosaceus*,<sup>4</sup> Dr. Carpenter further develops the theory that the axial cords "are really nerve-trunks, and that the five-chambered organ in the centrodorsal basin is their centre." He refers to the "quickness and consentaneousness" with which the coiling and uncoiling of the arms are effected, and to the fact that irritation of the oral pinnules causes the whole circlet of arms to close together

<sup>1</sup> Semper, "Kurze Anatomische Bemerkungen ueber Comatula," 'Arbeiten aus dem Zool. Zoot. Institut in Würzburg,' Band i, 1874, p. 259. Translated in the 'Annals and Magazine of Natural History,' 1875, p. 202.

<sup>2</sup> Perrier, 'Archives de Zoologie Expérimentale,' tome ii, 1873, p. 55.

<sup>3</sup> Carpenter, 'Annals and Magazine of Natural History,' 1875, p. 206.

<sup>4</sup> Carpenter, 'Proceedings of the Royal Society,' 1876, p. 226.

as strong evidence of the presence of a definite nervous system, and suggests that the histological simplicity of the axial cords may "be related to the fact that as the muscles are all flexors the nerves have only one function to perform, and that there is consequently no need of the insulation which they require where nerve-fibres of very different functions are bound up in the same sheath."

He further supports his theory by the following experiment made at Oban in 1867, and which for convenience of reference I shall describe as

Experiment A.—The entire visceral mass was removed from a living specimen so as to leave nothing but the calyx with the central capsule and its prolongations and the arms. A needle was then passed down the canal surrounded by the First Radials (cf. fig. 1) so as to irritate the chambered organ. "All the ten arms then suddenly and consentaneously closed up. On the withdrawal of the needle the arms gradually straightened themselves again, and again coiled up as before when the irritation of the central organ was renewed."

In January, 1876, Greef<sup>1</sup> called attention to the thickened epithelium forming the floor of the ambulacral grooves both of the arms and disc. He pointed out the close correspondence both in position and histological structure between this ambulacral epithelium of *Antedon*, and the radial nerves and circum-oral commissure of a Starfish, and suggested that the former, like the latter, was nervous in function. At the same time he denied the nervous character of the axial cords.

In the following month Ludwig,<sup>2</sup> without being acquainted with Greef's work, described for the first time a "delicate fibrillar band" immediately beneath the ambulacral epithelium, i. e. what we have named above the subepithelial band (fig. 2, *h*), which he regarded on histological and morphological grounds

<sup>1</sup> Greef, "Ueber den Bau der Crinoideen," 'Sitzungsb. d. Gesellsch. z. Beförd. der gesam. Naturwiss. zu Marburg,' No. 1, 1876, pp. 16—29.

<sup>2</sup> Ludwig, 'Nachrichten v. der Königl. Gesellschaft der Wissenschaften, und der Universität zu Göttingen,' No. 5, Feb. 23rd, 1876.

as the true nervous system of Antedon, and as the representative of the radial nerves of other Echinoderms.

In April of the same year P. H. Carpenter<sup>1</sup> confirmed Ludwig's description of the subepithelial bands which he had himself independently discovered, and agreed with him in regarding them as nervous. He also showed that the cord *x*, described by Semper, was not identical as he had supposed with Perrier's fibrous cord, and that neither of these structures corresponded to the subepithelial band, Semper's cord being merely a pigmented cellular thickening between the ambulacral and subtentacular canals, while Perrier's fibrous cord is a muscular band in the ventral wall of the ambulacral canal. P. H. Carpenter, however, differed from Ludwig in regarding not only the subepithelial bands, but the axial cords also as nervous, and he was the first to distinctly maintain the existence in Antedon of this double nervous system in spite of the morphological difficulties involved in this view. He brought forward as additional evidence in favour of the nervous character of the axial cords the fact that in the arms of *Actinometra* the cord enlarges in the centre of each ossicle and gives off branches to both dorsal and ventral surfaces, some of the latter reaching "the bases, or in some cases even the tips of the respiratory leaves." He even suspected a connection between some of these branches of the axial cord and the subepithelial bands; some, he says, "appear to enter into the plexus of tissue forming the organic base of the skeleton, others seem to become connected with epidermic structures."

In a supplemental note<sup>2</sup> Dr. Carpenter also confirms the existence of the subepithelial band, and considers that it is "by no means improbable, looking alike to its position and to its histological character, that this band is a nerve." On account mainly of its position he suggests that it is "an afferent rather than a motor nerve." He also brings forward the following extremely important additional experimental evidence

<sup>1</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," *Journal of Anatomy and Physiology*, April, 1876.

<sup>2</sup> Carpenter, 'Proceedings of the Royal Society,' vol. xxiv, 1876, p. 651.



in support of the nervous nature of the central capsule and axial cords.

Experiment B.—The visceral mass was removed from a large and vigorous Antedon, leaving the calyx with the central capsule and the arms intact. On replacing the animal in the water it executed the usual swimming movements as perfectly as the entire animal had previously done.

Experiment C.—From a second active specimen, the entire centrodorsal basin with its contents and appendages were removed. On replacing the animal in the water all the arms were rigidly straightened out, apparently by the action of the elastic ligaments which the muscles were powerless to antagonise.

Experiment D.—In an active specimen the soft parts of one of the arms were divided down to the calcareous segments. On replacing the animal in water all the arms worked as usual without the slightest disturbance of regularity.

Experiment E.—By means of nitric acid applied with a fine brush, the dorsal half of one of the arms was dissolved away until the axial cord was reached and destroyed. On replacing the animal in the water the injured arm remained rigidly stretched out, while all the other arms worked as usual.

From these experiments Dr. Carpenter concludes that the central capsule is the co-ordinating centre of a nervous system whose peripheral portion consists of the axial cords of the rays, arms, and pinnules; also that the subepithelial band, if a nerve at all, has no immediate relation to the swimming movements of the arms.

In 1877 Ludwig<sup>1</sup> published a more detailed account of the subepithelial band in Antedon, in which he describes the band and the columnar epithelium covering it as being sometimes directly continuous with one another and sometimes separated by a delicate horizontal lamella. This lamella he finds to be a more constant and evident structure in Antedon Eschrichtii

<sup>1</sup> Ludwig, 'Morphologische Studien an Echinodermen,' Heft i, Abh. i; 'Separat. Abdruck aus der Zeitschrift f. wissenschaftliche Zoologie,' Bd. 28.

than in *A. rosaceus*. He considers that the subepithelial band is alone to be regarded as the nerve, and points out that the close histological similarity between this band in Crinoids and the radial nerve of an Asterid, which latter, from the position of the eyes, must certainly be nervous, is a strong argument in support of his view. He also discusses the claim of the axial cords to rank as parts of the nervous system; but while admitting the great importance of Dr. Carpenter's experiments, considers that the case is not yet satisfactorily proved, and that the morphological difficulties involved in the possession by Crinoids of a nervous system altogether unknown in other Echinoderms, are too great to permit the acceptance of Dr. Carpenter's views. According to Ludwig, the axial cords are parts of the connective-tissue basis of the skeleton, which persist in an uncalcified condition, and are probably nutritive in function.

P. H. Carpenter, in a further paper on the arms of Crinoids,<sup>1</sup> and in a monograph on the genus *Actinometra*,<sup>2</sup> brings forward strong additional evidence in support of the nervous nature of the axial cords. He shows that in *Antedon rosaceus* the oral pinnules differ from the other pinnules, not only in being destitute of tentacles (as pointed out by Dr. Carpenter in 1865), but also in having no ambulacral groove, no thickened ambulacral epithelium, and no trace of the subepithelial band, i. e. that they are totally devoid of what Ludwig considers to be the sole nervous system of *Antedon*; and yet these oral pinnules are peculiarly irritable, a slight touch being sufficient to cause all ten arms to be suddenly coiled up over the disc.

He further finds that in *Antedon Eschrichtii* this absence of ambulacral groove and epithelium, and of the subepithelial band, occurs not only in the oral pinnules, but at the distal extremities of the arms and other pinnules. The allied

<sup>1</sup> P. H. Carpenter, "Remarks on the Anatomy of the Arms of the Crinoids," part ii, 'Journal of Anatomy and Physiology,' vol. xi, October, 1876.

<sup>2</sup> P. H. Carpenter, "On the Genus *Actinometra*," 'Transactions of the Linnean Society,' 2nd series Zoology, vol. ii, part i, 1879.

genus *Actinometra* is still more remarkable, for here entire arms may be completely devoid of ambulacral groove and epithelium, and of the subepithelial band, and yet such arms, though on Ludwig's theory possessing no nerves at all, are described on Semper's authority as exhibiting as regular and active movements while swimming as the other arms. On the other hand, the axial cords or their branches extend along all the arms and pinnules, whether possessing ambulacral grooves or not.

In all cases the absence of ambulacral grooves is associated with the absence of tentacles. Non-tentaculiferous arms are met with in a large number of species of *Actinometra*, no less than twenty-three out of the forty-eight species collected by the "*Challenger*"<sup>1</sup> having more or fewer of such arms, the number of which varies greatly in different individuals.

In a short paper published in 1883 Perrier<sup>2</sup> adopts very definitely the views of the Carpenters concerning the nervous system. He traces branches of the axial cords into connection, through the intermediation of stellate cells, with the muscle fibres. Other branches are traced by him into the tentacles. He gives no figures, however, and his descriptions leave some doubt as to whether the stellate cells do not rather belong to the connective-tissue investment of the nerve or muscle than to the nerves themselves.

P. H. Carpenter<sup>3</sup> has recently described tripolar cells intercalated in the course of the axial cords and their branches in *Antedon*. He has also traced in three species of *Antedon* a fibrillar plexus derived from the axial cords into the connective tissue of the perisomæ forming the ventral surface of the disc, and is "strongly inclined to believe that extensions of this plexus are in direct connection with the fibrils of the subepithelial bands."

<sup>1</sup> P. H. Carpenter, "Preliminary Report upon the Comatulæ of the '*Challenger*' Expedition," '*Proceedings of the Royal Society*,' No. 194, 1879, p. 395.

<sup>2</sup> Perrier, "Note sur l'organisation des Crinoides," '*Comptes rendus*,' tome xcvii, 1883, pp. 187—189.

<sup>3</sup> P. H. Carpenter, "Notes on Echinoderm Morphology," No. 6 '*Quarterly Journal of Microscopical Science*,' 1883.

Finally, Dr. Carpenter has very recently<sup>1</sup> given a summary of the investigations concerning the nervous system of the Crinoids which have been published since his former paper in 1876. He points out that the evidence accumulated in this interval is most strongly in favour of his view, which, on the other hand, is opposed merely "by a theoretical homology, a preconceived notion of what Crinoids ought to be." He concludes with some important observations on the morphological aspects of the question, which will be noticed in a later section of this paper.

The present position of the question may be briefly described thus. The Carpenters and Perrier, on the one hand, maintain that the central capsule and axial cords, with their branches, constitute the essential and principal part of the nervous system, both motor and sensory, while the subepithelial bands, if nervous at all, are of very subordinate functional importance. On the other hand, Ludwig and the German morphologists generally maintain that the subepithelial bands constitute the sole nervous system. The former school cite in support of their views a large mass of anatomical and histological observations and certain direct experiments; while the latter school rely entirely on theoretical morphological objections to the views of their opponents.

### III. EXPERIMENTAL INVESTIGATION OF THE NERVOUS SYSTEM OF *ANTEDON ROSACEUS*.

This section of the paper, containing the account of my own investigations made at Naples last April, I propose to subdivide under the following heads:—A. The movements of uninjured specimens. B. The effects of removal of the visceral mass. C. The power of regeneration. D. The functions of the central capsule. E. The functions of the axial cords. F. The functions of the subepithelial bands.

<sup>1</sup> Carpenter, "On the Nervous System of the Crinoidea," 'Proceedings of the Royal Society,' 1884.



### A. The Movements of Uninjured Specimens.

The normal position of *Antedon rosaceus*, the species on which all my experiments were made, is a fixed one, the animal being attached by the dorsal cirri to some foreign body, and the arms spread out horizontally with their tips slightly flexed. The oral pinnules are bent over the disc, crossing one another above it; the other pinnules are spread out nearly at right angles to the arms.

In an aquarium containing a large number of specimens the great majority will be found attached either to the bottom or sides of the tank, i. e. with the oral surface directed either upwards or more or less obliquely; some specimens, however, are almost certain to be found, if there be foreign bodies in suitable positions for attachment, inverted, with the oral surface downwards.

An *Antedon* when once attached exhibits very little tendency to alter its position, and may remain fixed in the same place for weeks. If detached, either spontaneously or by force, it can, and usually does, swim actively until it reaches a suitable place of rest, to which it anchors itself by its cirri. The normal swimming movements, which are peculiarly graceful, consist in strong flexion of the proximal half of the arm, which is raised vertically over the disc, and then extension of the whole arm, the distal half of which is thrown out something like a whiplash or the line of a flyrod. During flexion the pinnules are folded alongside the arm; during extension spread out so as to expose as great a surface as possible. Usually two or three arms are raised simultaneously, sometimes as many as five, and the only rule I have noticed is that the two arms of each pair are always flexed alternately and not simultaneously.

When attached by its cirri the arms of *Antedon* exhibit but very slight movements; they are usually spread out widely, apparently to expose as large a surface as possible for the entanglement of food particles, which, if they once come in con-

tact with the ambulacral epithelium, get carried by the action of its cilia to the mouth.

Irritation of the ambulacral groove at any part causes the adjacent pinnules to be at once turned forwards, i. e. with their tips towards the free end of the arm, and folded alongside the irritated part, apparently to protect it from further injury. Slight irritation of a pinnule or of an arm causes correspondingly slight and local movements; stronger irritation causes movements of the whole arm, which may spread to other arms, or lead to the animal detaching itself and swimming freely. Irritation of the oral pinnules, however slight, causes them to be firmly closed over the disc, and stronger or prolonged irritation causes the arms to be flexed strongly, so as to cover the disc, or else the whole animal to detach itself and swim away.

If an Antedon be detached and placed with its oral surface downwards, it will right itself almost at once. If the surface on which it is placed be a rough one, the righting movement is effected in a few seconds or almost instantaneously. In a glass vessel it takes longer to perform, but with an active specimen I have never seen more than two minutes spent over the operation. In righting itself an Antedon first flexes all the arms slightly, so as to raise the disc a little above the ground; then follows a moment of apparent uncertainty as to which arm to use. One arm is then flexed more strongly than the others, so as to slightly lift the disc on that side, the pinnules of the flexed arm being extended and apparently used to push against the ground. Then, after another pause, a rather sudden and violent flexion of the arms immediately adjacent to the already flexed one causes the animal to turn on its side, when a few energetic swimming movements place it right way up. An active animal has apparently the strongest objection to being placed mouth downwards, and will right itself again and again if so inverted. When attached by the cirri, however, they may, as noticed above, remain in the inverted position for days or weeks.

If an arm be cut off from an active Antedon, the detached

arm will retain its vitality for many hours. It will at first exhibit strong movements of flexion, lasting from a few minutes to as long as a couple of hours, the arm being alternately coiled up spirally, and then extended with great force and rapidity.

Antedon, if kept in captivity, requires the water to be frequently changed, or else very efficiently aerated. Specimens left over-night in a small basin of sea-water were found dead the next morning. In dead specimens, owing to the unopposed action of the elastic ligaments, the arms are very strongly extended.

#### B. On the Effects of Removal of the Visceral Mass.

In a living specimen the visceral mass can be removed from the calyx with great ease, as was pointed out long ago by Dr. Carpenter. If the visceral mass be grasped with forceps an exceedingly slight pull suffices to remove it. In such eviscerated specimens the central capsule with its prolongations and the axial cords remain in the calyx intact, excepting, of course, the branches of the cords described by P. H. Carpenter as distributed to the oral perisome; the ambulacral grooves and other soft parts, on the other hand, are torn across at the bases of the arms, and the subepithelial bands consequently isolated from one another.

Experiment 1.<sup>1</sup>—A large and vigorous specimen was eviscerated without removal from the water. On being released it remained quiescent for about a minute, and then swam about the tank actively and in a perfectly normal manner. After a short time it came to rest on the bottom in a perfectly normal position. Half an hour later, without the slightest disturbance or irritation of any kind, it began spontaneously to swim again actively and normally. Coming in

<sup>1</sup> For convenience of reference I propose to number the various experiments consecutively. It will be understood that they were not made in the order given here, and that only those which seem of distinct importance are recorded. No experiment is described from a single observation only, and in most cases the experiments were repeated several times.

contact with a piece of stick, it attached itself to it by the dorsal cirri, and remained there for more than a week.

The above experiment is the same as Dr. Carpenter's Experiment B described above. It is extremely important as proving that the co-ordinating mechanism which regulates the complex swimming movements of the arms is entirely without the visceral mass. As the direct connection between the sub-epithelial bands of the several arms is also destroyed, the experiment renders it extremely doubtful whether these bands have any part in regulating the swimming movements of the arms.

Experiment 2.—An active specimen was eviscerated, and allowed to come to rest. The ventral surface of one of the arms was then irritated gently with a needle; active movements both of the irritated arm and of the others resulted. The same effect followed irritation of one of the ordinary pinnules; while irritation of the oral pinnules caused immediate and strong flexion of all the arms.

This shows that the effect of irritation of the arms or pinnules is practically unmodified by the removal of the visceral mass; the only difference I have noted being that the response is slightly quicker and more extensive in an eviscerated than in an uninjured specimen. The nervous connection between the sensory epithelium of any one of the arms or pinnules and the muscular system, not only of that arm, but of all the others as well, must, therefore, be without the visceral mass.

As a source of irritation in this and other experiments I employed at first scratching with a sharp needle. I found afterwards that nipping with forceps was preferable, as the needle is apt to shake the whole animal, and so cause disturbance of parts other than those it is desired to irritate. The nip should be a sharp sudden one, and the irritated part released at once. In all the experiments here recorded, except



when otherwise specified, both needle and forceps irritation were tried. In some instances the application of acid by a fine brush was made use of as an irritant; but this can only be done satisfactorily on specimens removed from the water.

Experiment 3.—An active specimen was eviscerated and allowed to come to rest in the normal position. It was then inverted and placed mouth downwards on the bottom of the tank. After a short rest it righted itself in the normal manner, but rather more slowly than usual, the interval between inversion and completion of the righting manœuvre being about two and a half minutes. This experiment was repeated many times with different specimens. Some righted themselves instantaneously, others took a longer or shorter time, but the general average of the times taken by eviscerated specimens to right themselves was about half a minute longer than that of uninjured ones.

This affords strong additional evidence that the co-ordinating centre of the complex muscular movements of which an Antedon is capable is situated not in the visceral mass, but in the calyx.

#### C. On the Power of Regeneration of Eviscerated Specimens.

It has been stated above that an eviscerated Antedon not only attaches itself by its cirri in a perfectly normal manner, but that it may remain so attached for a week or more. On experimenting one day with a specimen that had been eviscerated about a fortnight previously, I noticed that it righted itself when inverted rather more readily than is usual in eviscerated specimens; and on examination I found that very considerable regeneration of the visceral mass had occurred. The soft tissues lining the calyx were of some thickness; a mouth was already present in the centre of the oral surface, and ambulacral grooves had formed converging from the arms to the mouth. I at once took steps to secure a complete

series of specimens, showing all stages of this regeneration, and I hope to be able shortly to describe the process in detail.

That *Antedon* possesses this very extensive power of regeneration, greatly exceeding even that of *Holothurians*, was an entirely new fact to me. Dr. Carpenter tells me that he was led to suspect this long ago, and he has very kindly shown me specimens that have been in his possession for many years, which seem to me to be clearly cases in which regeneration has been partially effected. Dr. P. H. Carpenter also tells me he has known this fact for some time, though I believe no notice of it has yet been published. It is only fair to add that while at Naples the possibility that an eviscerated *Antedon* might regenerate its visceral mass was suggested to me in conversation by Dr. Örley, of Buda-Pesth. I made very light of the suggestion at the time, and was much astonished when a few days later I found the specimen described above.

The influence of the nervous system on the regeneration of lost parts is a point concerning which we know very little; but the apparent ease with which this extensive regeneration is effected in *Antedon* would certainly be still more surprising were the main centre of the nervous system to be lodged in the part lost, and so far may be regarded as an argument against such a location.

#### D. On the Functions of the Central Capsule.

Experiment 4.—A specimen was eviscerated and allowed to come to rest; a needle was then passed from the oral surface down the canal surrounded by the First Radials (fig. 1) so as to irritate the central capsule; the result was immediate flexion of the arms, and in many cases active swimming movements of the whole animal.

Experiment 5.—A specimen was eviscerated and then cut into two parts, one having two pairs of arms and the other three. The central capsule, which was divided and freely exposed by the operation, was then irritated by a needle. The

slightest irritation caused very active and violent flexion of the arms.

Experiment 6.—An active uninjured specimen was held under water, and the dorsal half of the centrodorsal plate removed by a single snip with a large pair of scissors so as to expose and partly remove the central capsule (cf. fig. 1). On being released the animal fell to the bottom with the arms very strongly extended, but in about twenty minutes gradually righted itself and assumed the normal position. The exposed central capsule was then irritated, first with a needle and then with strong nitric acid applied by a small brush; the effect of irritation was to cause very strong and spasmodic flexion of the arms, which in the first case ceased on removal of the stimulus, but in the case of the acid persisted for several hours.

The three preceding experiments show that irritation of the central capsule, whether mechanical or chemical, causes strong flexion of all the arms, which persists as long as the stimulation is continued. Experiment 4 is the same as Dr. Carpenter's Experiment A, though the results are not quite identical; for while Dr. Carpenter describes sudden and consentaneous flexion of the arms as following irritation of the central capsule from the oral surface, I have found that swimming movements quite as often result. The difference is a slight one, and may, I believe, be accounted for by the oral pinnules being accidentally irritated in some of the experiments. If these were clipped off I found that swimming movements of the arms almost invariably followed irritation of the central capsule from above.

The experiments prove in the most positive manner that the central capsule is in direct physiological connection with the muscles of the arms; and the further fact that the experiments yield identical results, whether performed on eviscerated or on unmutilated specimens, proves that the subepithelial bands form at any rate no part of the central mechanism.

Experiment 7.—The centrodorsal plate of an active

specimen was removed with scissors and the central capsule carefully scooped out with a small scalpel. The animal on being released fell to the bottom of the water, where it lay on its side with the arms very strongly extended; it remained in this position for several hours without any attempt to move. If taken from the water and thrown in again the arms moved fairly actively, but there was no attempt at swimming, each arm apparently acting quite independently of the rest. Finally, if placed on its oral surface it remained there for an indefinite time without making the slightest attempt to right itself.

Experiment 8.—The preceding experiment was repeated on an eviscerated specimen, the results being in all respects the same.

These two experiments are of very great importance. They show that removal of the central capsule completely destroys the co-ordinating mechanism between the arms as tested (a) by the power of executing the normal swimming movements, (b) by the power of righting itself when inverted; both these powers being permanently destroyed by the operation. To obtain definite results it is necessary to completely remove the central capsule, and this I have found cannot be effected by simply cutting away the centrodorsal plate; besides this the capsule must be either scraped out with a fine scalpel or else destroyed by free painting with strong acid. Specimens in which the centrodorsal plate has been simply snipped off, though they lose temporarily the power both of swimming and of righting themselves, yet regain these more or less completely after an interval of half an hour to an hour. If, however, sufficient care has been taken to entirely destroy the central capsule the loss of power is absolute and permanent.

Experiment 9.—The centrodorsal plate of an active specimen was removed, and the central capsule entirely destroyed; the cavity was also very freely painted with nitric acid so as to expose and destroy the pentagonal commissure connecting the axial cords together at their roots (cf. fig. 3). After being left



at rest for an hour the arms were irritated one by one; each arm responded readily and extensively to the stimulation, but the movement was limited to the arm directly irritated, none of the other arms sharing in it, except sometimes the other arm of the pair to which the irritated arm belonged.

This experiment shows that the physiological connection between the arms can be destroyed by removal of the central capsule and of its branches including the pentagonal commissure. After this operation the several arms, with the exception of the two of each pair, are physiologically isolated from one another. The experiment yields identical results whether the visceral mass be present or not.

I have found it very necessary after severe operations to allow sufficient time for recovery from shock before experimenting further, and through failure to observe this precaution I obtained at first several very contradictory and perplexing results. From half an hour to an hour I usually found to be sufficient.

#### E. On the Functions of the Axial Cords.

Experiments on the functions of the axial cords and their branches fall naturally under two heads, i. e. those concerned with the relations of these structures to sensation and to motion respectively.

I propose to commence with the former of these, though, as it sometimes happens that the same experiment is concerned with both sensation and motion, it will not be advisable to draw too sharp a line between the two divisions.

Experiment 10.—Various parts of the surface, both of the disc and the arms, of active uninjured specimens were irritated, both mechanically and chemically, in order to determine the normal distribution of sensation. All parts of the surface were found to be sensitive, but in very unequal degrees. Irritation of the dorsal surface of the calyx caused only slight

movements of the arms, unless the irritation were severe or prolonged. Irritation of the dorsal or lateral surfaces of the arms, where the layer of integument is very thin, caused flexion of the arms, with extension of the pinnules close to the irritated spot. The response was usually ready, but the movement only slight. Prolonged or more violent irritation caused exaggeration of the movement, together with approximation of the adjacent arms towards the irritated arm, as though to remove the source of irritation, and in some cases active movement of the whole animal in a direction away from the irritated arm. Irritation of a pinnule causes, according to the degree and duration of the stimulation, movement of the pinnule, movement of the whole arm, approximation of the adjacent arms to the affected one, or active movement of the whole animal away from the source of irritation. Irritation of the oral pinnules causes, as already noticed, immediate and very active flexion of all the arms, so as to close in over the disc.

The epithelium of the ambulacral grooves is extremely sensitive, and the results of stimulation are very definite. The slightest irritation causes instantaneous movement of the four or five pairs of pinnules immediately adjacent to the irritated spot, the pinnules being folded alongside the ambulacral groove so as to close it in and grasp the needle or other source of irritation. If the stimulation be continued the arm is actively flexed and the adjacent arms applied to it, and rubbed along the affected part, as though to remove the source of irritation. Finally, irritation of the ventral surface of the disc between the ambulacral grooves causes movements of the arms, but not nearly so active as when the oral pinnules are touched.

Experiment 11.—An active specimen was eviscerated, and left for half an hour. The calyx, arms, and pinnules were then successively stimulated, as in the preceding experiment. The results were exactly the same, showing that the communication between the sensitive surface of any part of the calyx, arms, or pinnules, and the motor mechanism of all the arms, is placed elsewhere than in the visceral mass.

**Experiment 12.**—An active specimen was taken, and all the soft parts scraped away with a knife from the ventral surface of one of the arms, the scraped portion being about a quarter of an inch in length and one inch from the disc. The pinnules were immediately folded closely alongside the wound, and the animal on being released swam actively in a direction away from the injured arm. It soon came to rest in the normal position, and about six minutes after the operation the distal end of the injured arm was nipped with the forceps. The distal part of the arm, beyond the injury, was at once flexed actively, the proximal part less actively, and the other arms did not move. After a twenty minutes' interval the distal end of the injured arm was again nipped, when active movement of all the arms at once resulted, the animal moving rapidly away from the source of irritation.

The above experiment shows that the communication between the sensitive surface of an arm or pinnule and the motor mechanism of all the arms is not effected by the subepithelial band. The practically negative result obtained when the stimulation was applied very shortly after the operation is, I think, most certainly to be ascribed to the shock of the operation, which, as already noticed, must always be kept in mind as a disturbing element.

If the communication is not effected by the subepithelial band, nor by any of the soft parts of the ventral surface of the arm—all of which were scraped away in the operation—it must take place either through the integument of the dorsal and lateral surfaces of the arm, or through the calcareous segments, or through the axial cords, for these are the only parts left uninjured. To determine which of these is the real path of communication the following crucial experiment was made.

**Experiment 13.**—A large and vigorous specimen was taken, and a quarter of an inch of one of the arms, about an inch from the base, thoroughly scraped with a scalpel all round so as to remove the soft parts as completely as possible. The pinnules

of the affected part and for a quarter of an inch on either side of the wound were cut away to prevent any possibility of contact communication between the parts on either side of the injury. The injured part was then painted all round very freely with strong nitric acid, the operation being repeated until fully half the thickness of the calcareous segments had been dissolved away. The wound was then washed freely with sea water and the animal returned to the tank. It fell at once to the bottom on its side with the injured arm and the other one of the pair stretched straight out horizontally, and the other arms rather strongly extended. After a few minutes it began to move slowly, and in six minutes had completely resumed the normal position. After half an hour's interval the distal end of the injured arm was sharply nipped with forceps, when strong active movements of all the arms at once resulted, the animal moving rapidly away from the source of irritation.

The above experiment, which was repeated several times, both on entire and on eviscerated specimens, proves conclusively that the communication between the distal end of the irritated arm and the motor mechanism of the arms is effected by the axial cord; in other words, that the axial cord plays the part of an afferent or sensory nerve, conveying impulses centripetally. Furthermore, that it is the normal path of communication of such impulses is, I think, evident from the response to stimulation being as ready when it alone remains as in the uninjured animal. It remains, however, to show whether it is the only path of communication. To test this I attempted several times to divide the axial cord between two of the segments by a fine scalpel, but I failed, as Dr. Carpenter had done previously, owing to the fact that as soon as the knife reached the axial cord the arm was at once thrown off, usually at a point two or three segments nearer the disc than the injury. I then tried the plan adopted by Dr. Carpenter,<sup>1</sup> i. e. burning away the dorsal half of the arm with nitric acid so as to expose and divide the axial cord, and with the following results.

<sup>1</sup> Carpenter, 'Proc. Royal Soc.,' 1876, p. 654.



Experiment 14.—An active specimen was removed from the water, the dorsal surface of one of the arms carefully dried, and strong nitric acid applied with a fine brush to the dorsal surface of the sixth and seventh radials, which were dissolved away until the axial cord was exposed and destroyed. If the arm were held during the operation it was usually thrown off, but if the disc only were held and the arm allowed merely to rest on the fingers, the operation was always successful. The animal was then returned to the water, where it assumed almost at once the normal position. After half an hour's rest, the distal end of the injured arm was nipped sharply with forceps; active movements of the irritated arm beyond the injury ensued, but no movement whatever of either the proximal part of the injured arm or of any of the other arms.

This experiment also was repeated several times on both entire and eviscerated specimens, the results being without exception as recorded above. It is difficult to limit the action of the acid to the dorsal surface of the arm, but by sufficient care it can be done, and on several occasions the ambulacral epithelium, including of course the subepithelial band, was left absolutely uninjured, responding to stimulation in a perfectly normal manner. The experiment must, I think, be considered, when taken in conjunction with Experiment 13, as proving that the axial cord is the sole afferent communication between the arm and the central motor mechanism, for the former experiment shows that the communication is still perfect when it alone remains, while the latter shows that division of the cord, other parts remaining intact, destroys the communication absolutely.

Experiment 15.—One further and very obvious experiment is worth recording. One of the arms of an active specimen was cut across about its middle, and the animal held in the tank so that the stump of the amputated arm was just above the surface of the water; the cut end of the axial cord could then be very readily seen with the naked eye. The

stump was carefully dried and the axial cord touched with a fine needle, or with a finely-pointed brush charged with nitric acid, very violent movements of all the arms at once resulting. Similar stimulation of the ambulacral epithelium or of other parts of the section produced but very slight and local movements.

This concludes my experiments as to the afferent functions of the axial cord, excepting certain points relating to the commissural connections between these cords, which will be dealt with later on. I propose now to inquire into the motor function of the axial cords.

Experiment 16.—As in Experiment 12, the soft parts were scraped away from the ventral surface of about a quarter of an inch of one of the arms, an inch from its base. On being returned to the water the animal swam actively, all the arms moving vigorously and normally, including the injured one, which, however, was rather less active than the others, and a little stiff at the scraped part, probably from direct injury to the muscles.

This experiment, which was repeated on eviscerated specimens with identical results, shows that the path by which motor impulses are conveyed to the muscles of the arms is neither the subepithelial band nor any part of the soft structures on the ventral surface of the arm.

Experiment 17.—The operation was the same as in Experiment 14, the dorsal half of one of the arms, about an inch from the disc, being dissolved away by nitric acid until the axial cord was exposed and divided. The animal was then returned to the water, where it remained quiescent for a few seconds, and then commenced to swim actively and spontaneously, all the arms moving perfectly normally, except the injured one, the proximal end of which moved slightly, while the distal part beyond the injury was perfectly motionless and

flexed spirally into a coil. After a short time the animal came to rest in a perfectly normal position, but for the spiral coiling of the distal part of the injured arm, which persisted. After a quarter of an hour's rest one of the uninjured arms was irritated, causing at once active movements of the uninjured arms and of the proximal part of the injured arm, but none whatever of its distal part.

Experiment 18.—In a fresh Antedon two injuries, similar to that in Experiment 17, were made in one of the arms at spots about an inch and a half apart. Stimulation of the arm itself, or of the pinnules, between the two wounds caused movements of the middle portion of the arm, but none whatever of the proximal or distal portions.

The two preceding experiments show that division of the axial cord destroys the motor communication between the parts on either side of the section as completely as we have already found it to destroy the afferent or sensory communication. When combined with Experiment 16, which shows that the motor communication is not effected by any of the other soft parts, the inference is irresistible that the sole motor communication is that afforded by the axial cords. One additional experiment may be mentioned in support of this conclusion.

Experiment 19.—One of the arms of a vigorous specimen was amputated by a snip of the scissors. The detached arm exhibited extremely active movements for about a quarter of an hour, coiling and uncoiling with great force and rapidity. After a time it became quiescent. It was then held in the tank with the proximal end just out of water. The end was carefully dried and the exposed section of the axial cord touched with a needle and with a fine brush charged with nitric acid. The slightest irritation, whether mechanical or chemical, caused violent and repeated flexion of the arm. Stimulation applied to other parts of the cut end produced but very little effect.

It still remains to inquire into the functions of the commissural

bands which connect the axial cords together, for if the axial cords are really nerves these connecting bands, which are identical with them in histological structure, must be nerve also, and experiment ought to throw light on their purpose. These commissures are of two kinds (cf. fig. 3): there is, firstly, the great pentagonal commissure in the First Radials which connects together the roots of the radial cords; and, secondly, we have in each Third Radial a rather complicated connection, by means of a transverse commissure and a chiasma, between the two axial cords into which each radial cord divides. I propose to deal with these two sets of fibres separately, taking the great pentagonal commissure first.

Experiment 20.—A specimen was eviscerated, and a needle passed down from the oral surface into the chambered organ, and worked about so as to destroy as completely as possible the central capsule and chambered organ (cf. fig. 1). The animal was then returned to the water, and left at rest for half an hour. One of the arms was then suddenly nipped with forceps, when all the arms exhibited active movement, though the animal did not attempt to swim.

This experiment shows that the central capsule does not form the sole physiological connection between the axial cords (nerves) of the several arms. Figs. 1 and 3 show that the pentagonal commissure, which is lodged in the First Radials, would not be touched by the operation, and, as it furnishes an anatomical connection between the axial cords, it was naturally suspected to be the physiological connection as well. To test this the following experiment was made:

Experiment 21.—The same specimen employed in the preceding experiment was taken, and the inside of the canal surrounded by the First Radials freely painted with nitric acid, until the pentagonal commissure was exposed and destroyed. The animal was then returned to the water and left for half an hour on its oral face, where it remained without any attempt to right itself or to swim. The arms were then strongly nipped



with forceps one by one; each arm when irritated responded by active movements, but none of the other arms stirred except the other arm of the pair to which the irritated arm belonged, which moved sometimes slightly, sometimes actively.

This last observation shows that there is a physiological connection between the two arms of each pair still remaining after the several pairs are isolated from one another by destruction of the pentagonal commissure. There is, as we have seen, an anatomical connection in the Third Radial (fig. 3), and the following experiments were made to test whether this furnishes also the physiological connection in question.

Experiment 22.—A pair of arms was cut off a specimen, the section passing between the First and Second Radials. After half an hour's interval one of the arms was stimulated, when both arms moved actively.

Experiment 23.—Another specimen was eviscerated and a pair of arms removed, the section passing between the Second and Third Radials (cf. fig. 3). All the soft parts were scraped from the basal portions of the arms, the basal pinnules were cut off, and the Third Radial and basal joints of the arms freely scraped and painted with nitric acid, so that the sole connection between the two arms was through the substance of the Third Radial. After half an hour one of the arms was sharply nipped; the irritated arm moved freely, and the other arm slightly but distinctly. The experiment was repeated with a second specimen, and an interval of three hours allowed between the operation and stimulation of the arm. In this case active and extensive movements of both arms followed on irritation of either one.

As the radial cord (fig. 3) divides into the two axial cords before entering the Third Radial, the sole anatomical connection between the axial cords of the two arms in the above experiment is afforded by the transverse commissure and the chiasma,

one or other of which, or both, must therefore furnish the physiological connection which the experiment proves to exist. From the anatomical relations of the parts, and from the fact that the proximal ends of the chiasma must almost certainly have been injured in the operation, I think it probable that the transverse commissure is the real connecting link in this instance. As to the chiasma, the disposition of the fibres suggests that it may be connected with the alternating movements of the two arms of each pair which we have seen to occur in the act of swimming.

#### F. On the Functions of the Subepithelial Bands.

The subepithelial bands are supposed by Ludwig, as we have seen above, to constitute the sole or main nervous system of *Antedon*. The experiments detailed above demonstrate the incorrectness of this view. They show that the central connection of the subepithelial bands on the oral disc is in no way essential to, in fact, has nothing whatever to do with the complicated and co-ordinated movements of swimming, and of righting when inverted; they show, further, that division or destruction of the subepithelial band at any place does not destroy or even disturb either the sensory or motor communications between the parts on either side of the injury. In fact, they not only prove conclusively that these structures are not the sole nervous system, but even raise doubts as to whether they belong to the system at all.

I think, however, that the close histological resemblance between the subepithelial bands and the axial cords, coupled with the close correspondence as regards their relations to the ambulacral epithelium which exists between Crinoids and other Echinodermata in which, as in Asterids, they are most certainly nervous, must compel us to consider these bands in *Antedon* as nervous in nature, though what their exact function is has yet to be determined. The ambulacral epithelium is extremely and exceptionally sensitive, and irritation of it is responded to in a definite and peculiar manner, i. e. by the

sudden folding of the pinnules alongside the irritated spot. The ambulacral grooves are structures of great importance to the animal, for it is by them that food particles are captured and swept along by the ciliary currents to the mouth. Furthermore, the subepithelial band is in very intimate relation with that most characteristic Echinoderm system, the ambulacral vessels and their prolongations forming the tentacles.

Seeing, then, that there are along the ventral surface of the arms structures of great importance in very close anatomical relation with these subepithelial bands, which agree histologically with what are undoubtedly nerves, it seems probable that these bands form a special part of the nervous system connected with one or other, or perhaps all of these special structures.

That the connection between the subepithelial bands and the ambulacral epithelium and tentacles is a very intimate one is shown by P. H. Carpenter's observation, alluded to above, that in both *Antedon* and *Actinometra* all three structures disappear together, both in the oral pinnules and in those arms or portions of arms which are devoid of ambulacral grooves.

### G. Summary of Results.

1. The central capsule and its prolongations, the axial cords and their branches, constitute the main nervous system of *Antedon*.

2. The central capsule is specially connected with the complex co-ordinated movements of swimming and of righting when inverted.

3. The axial cords act as both afferent and efferent nerves.

4. The subepithelial bands are probably also nerves, but their exact function, probably a special and subordinate one in connection with the ambulacral tentacles and epithelium, is not yet ascertained.

5. Evisceration apparently causes but little inconvenience to the animal, and the visceral mass is regenerated completely in a few weeks' time.

These results are in complete accordance with the views so steadfastly advocated for many years past by the Carpenters, and recently adopted by Perrier, while, on the other hand, they are in direct opposition to the tenets of the German school.<sup>1</sup>

#### IV. Morphological Considerations.

Certain points of very considerable morphological interest arise in connection with the results detailed above, and I propose in this concluding section to notice briefly a few of the more important of these.

In the first place the morphological difficulty arising from the possession by *Antedon* of an antambulacral in addition to the typical ambulacral nervous system of Echinoderms must be considered. This objection has been strongly urged by Ludwig, and constitutes indeed the real ground of his dissent from Dr. Carpenter's views; and it must be admitted that the presence of a complicated nervous system in Crinoids, which is apparently altogether unrepresented in other Echinoderms, is a feature which a morphologist might well shrink from accepting until the fullest proof was forthcoming. This proof I have attempted to supply in the preceding section; the morphological puzzle however, still remains to be considered.

Tiedemann<sup>2</sup> was the first to describe the ambulacral nervous system of Echinoderms, and since his time the five radial bands with their connecting circumoral commissure have been universally accepted as constituting the typical Echinoderm nervous system. This nervous system, as was pointed out by Tiedemann, is differently situated in the different groups:

<sup>1</sup> Since this paper was written Dr. Jickeli, of Jena, has published an account of experiments made on the nervous system of *Antedon*, which led him to strongly uphold the correctness of Dr. Carpenter's views. Many of Jickeli's experiments are identical with the ones described above, and his paper, ('*Zoologischer Anzeiger*,' 23rd June, 1884), although at present incomplete, contains much valuable information.

<sup>2</sup> Tiedemann, '*Beobachtungen ueber das Nervensystem und die sensiblen Erscheinungen der Seesterne*;' Meckel's, '*Archiv für Physiologie*,' Bd. i, 1815: and '*Anatomie des Rohrenholothuries, des Seesterns und Steineigels*,' Landshut, 1816.



in Asterids it is quite superficial, while in Ophiurids, Echinids, and Holothurids it is much more deeply placed, being separated from the surface by a thick layer of cutis which in the two former groups is firmly calcified. Agassiz<sup>1</sup> urged this difference of position as an objection to the homology of the radial bands in Asterids and Echinids, but the objection was not sustained. More recent researches, while confirming the presence and the nervous nature of these radial bands and oral commissure, and adding much to our knowledge of their minute structure and relations,<sup>2</sup> have, however, tended to show that they only form a part of what is really a very widely-spread and diffuse nervous system.

Thus in Asterids it is very easy to demonstrate that the nerve-layer, which is perfectly continuous with the epidermis, of which indeed it forms the deepest stratum, is not confined to the floor of the ambulacral groove, but extends; though as a thinner layer, over the tube feet.<sup>3</sup> The nervous layer can also be recognised with little difficulty in the epidermis of the dorsal or antambulacral surface, and Hamann<sup>4</sup> has shown that it really forms a continuous sheath over the whole dorsal surface of the animal, which though exceedingly thin over the greater part of the back, thickens considerably at certain places, notably at the bases of the respiratory processes. Hamann describes the epidermis of Echinoderms as consisting of elements of four kinds—(1) supporting cells; columnar cells, whose deeper ends are produced into fibres which pass down into the underlying dermis; (2) sensory cells; columnar and sometimes ciliated cells whose deeper ends are continuous with (3) the nerve-fibrils; delicate bands whose direction is mainly parallel to the

<sup>1</sup> Agassiz et Desor, "Catalogue raisonné des familles des Echinodermes," 'Annales des Sciences Naturelles,' 1846.

<sup>2</sup> Ludwig's researches on the nervous system of Echinoderms, embodied in his 'Morphologische Studien an Echinodermen,' are of especial value and importance; and a recent paper by Hamann, referred to below, contains many new points of great interest.

<sup>3</sup> I believe Greef was the first to show this in 1871.

<sup>4</sup> Hamann, "Beiträge zur Histologie der Echinodermen," 'Zeitschrift für Wissenschaftliche Zoologie,' Bd. xxxix, 1883.

surface of the epithelium and which are in places aggregated into bundles; and (4) ganglion-cells; small nucleated cells connected with the nerve-fibrils. Of these structures the two latter form the nervous elements, which in Asterids are directly continuous with the more superficially placed columnar cells.

In Ophiurids the radial nerves, though having the same histological structure as in Asterids, are completely distinct from the epidermis, and separated from it by a thick layer of calcified dermis. Here also, however, branches from the radial nerves can be traced into the tube feet, where they form a layer immediately beneath the epidermis. Whether an epidermic nerve-sheath or plexus is present on the ambulacral surface has not, I believe, yet been demonstrated. I have been led to suspect the existence of such a sheath on physiological grounds, but have not yet seen it.

According to Baudelot,<sup>1</sup> in *Ophioderma longicauda* each of the nerves to the tube feet gives off a branch, which "se portait en haut et en arrière, et m'a paru se perdre dans la région dorsale du bras."

In Echinids Krohn<sup>2</sup> was the first to show that the radial nerves, which, like those of Ophiurids, are separated from the external epidermis by a thick layer of calcified dermis, give off branches, which accompany the tube feet through the pores in the ambulacral plates, and run in the substance of the tube feet as far as their free ends.

Lovén<sup>3</sup> describes the branches which accompany the tube feet as spreading out on the external surface of the test to form a network of fibres with numerous ganglion-cells. He figures a part of this external nerve plexus in *Brissopsis lyrifera*, and says concerning it: "On conçoit que tous les rameaux

<sup>1</sup> Baudelot, "Etudes Générales sur le Système Nerveux," 'Archives de Zoologie Expérimentale,' tome i, 1872, p. 208.

<sup>2</sup> Krohn, "Ueber die Anatomie der Nervensystem der Echiniden und Holothuriën," 'Archiv für Anatomie,' 1841, translated in 'Annales des Sciences Naturelles,' tome xvi, 1841.

<sup>3</sup> Lovén, "Etudes sur les Echinoidées," 'Kongl. Svenska Vetenskaps Aca-  
demiens Handlingar,' Bandet ii, No. 7, Stockholm, 1874, p. 8, and pl. ii, figs. 30 and 31.

du tronc nerveux se divisant de cette manière, il y aura, répandu à la surface du corps, un système nerveux périphérique extrêmement développé, fournissant des nerfs aux radioles, aux pédicellaires, aux clavicles des fascioles, et en général à toutes les parties externes."

Fredericq<sup>1</sup> was led, from a series of experiments on *Echinus* and *Toxopneustes*, to suspect the existence "d'un plexus nerveux situé dans l'épaisseur de la peau qui recouvre le test à l'extérieur," but did not succeed in demonstrating its existence anatomically.

More recently Romanes and Ewart<sup>2</sup> have described experiments on living *Echini*, which lead them to believe in the existence not only of an external nerve plexus outside the test, but also of an internal plexus on its inner surface; they further believe that the two systems are connected by nerve-fibres running through the plates of the test. The external plexus they figure<sup>3</sup> and describe "as lying almost immediately under the surface epithelium, and extending from the shell to the spines and pedicellariæ;" and in a postscript they state that they "have been successful in obtaining full histological demonstration of the internal nervous plexus of *Echinus*," and promise full descriptions of "its character, distribution, and mode of communication with the external plexus."<sup>4</sup>

Concerning *Holothurids*, both Krohn and Baudelot describe, in the memoirs cited above, branches from the radial nerves to the tube feet. More recently Hamann,<sup>5</sup> in the paper already quoted, has added valuable details concerning the distribution of these branches. He shows that the branches to the tube

<sup>1</sup> Fredericq, "Contributions à l'étude des Echinides," 'Archives de Zoologie Expérimentale,' tome v, 1876, p. 438.

<sup>2</sup> Romanes and Ewart, "On the Locomotor System of Echinodermata," 'Phil. Trans.,' 1881, part iii.

<sup>3</sup> Romanes and Ewart, loc. cit., p. 836, pl. 80, figs. 16—18. These figures are very different to Lovén's, which, however, were drawn from another genus.

<sup>4</sup> Loc. cit., p. 882.

<sup>5</sup> Hamann, loc. cit., p. 168, and pl. ii, figs. 51, 52, and 53.

feet, which are at first situated, like the radial nerves from which they arise, beneath the dermis, soon pass through this, and expand to form nerve sheaths around the tube feet and immediately beneath the external epidermis.

From the above descriptions it follows that the ordinary text-book accounts of the Echinoderm nervous system, which mention the radial nerves and the circumoral commissure, but nothing more, require very considerable modification.

We have in addition to the Crinoids four well-marked groups of recent Echinoderms, the Asterids, Ophiurids, Echinids, and Holothurids. Of these four there is I think no doubt that the Asterids must be regarded as the most primitive group, while the apodous Holothurids are perhaps the most modified. This primitive character of the Asterids is well illustrated by their nervous system, which as we have seen above is in the form of a continuous nerve-sheath enclosing the whole body, and directly continuous with the external epidermis of which it forms the deepest layer. This nerve-sheath is thickened at certain places, notably along the ambulacral grooves, where it forms the five radial or ambulacral nerves. Such a condition of the nervous system there is very strong reason for regarding as a very primitive one. It occurs in a slightly modified form in many Cœlenterates; it occurs in that primitive group of Nemertines which Hubrecht proposes to call Palæonemertini; it occurs also in the young of *Sagitta* and in several other cases. Even in Vertebrates the central nervous system really remains throughout life continuous with the epidermis, for the epithelium lining the central canal of the cord and the ventricles of the brain, was originally part of the surface epidermis.<sup>1</sup>

The fact that the Asterid nerve system remains in this primitive condition is of considerable importance from two points of view; in the first place it shows us the parent form from which the more modified nervous systems of other Echinoderms must have sprung, and thereby throws great light on

<sup>1</sup> Attention has recently been directed to this point by Sedgwick in the 'Proceedings of the Cambridge Philosophical Society,' vol. iv, pl. vi.



the mutual relations of these several forms ; in the second place it is of special interest in connection with the subject of the present paper, as showing that the Asterids are in at any rate one extremely important respect far more primitive than the Crinoids. I propose to say a few words on both these points.

Starting with the Asterid nervous system it is easy to derive from it, theoretically, the nervous systems of other groups. The sinking down of the radial nerves in Ophiurids and Echinids may possibly be connected with the development of the protective calcareous plates on the ambulacral surface, while the similar position they hold in Holothurids is probably due to the descent of this group from mailed ancestors provided with calcareous ambulacral plates, a line of descent for which there is a considerable amount of evidence forthcoming. That the radial nerves of Ophiurids, Echinids, and Holothurids are really the same things as the radial thickenings of the nerve-sheath in Asterids, in spite of their difference of position, is practically proved by the identical relations of the branches of these nerves or thickenings to the tube feet, which branches in all cases alike form sheaths immediately beneath the epidermis. The external plexus of Echinids may clearly be viewed as a somewhat modified nerve-sheath ; and the internal plexus of Romanes and Ewart, which is said to be connected directly with the external plexus through the substance of the test, may be explained as due to this nerve-sheath having commenced to shift inwards, just as the radial nerves have done, but at present remaining entangled in the substance of the calcified dermis.

As regards the origin of the Crinoid nervous system, I think that the Asterid again gives us an important clue, though much yet remains to be explained. It is commonly assumed that the subepithelial bands of the Crinoid are homologous with the radial nerve-bands of an Asterid, and I think the homology must be accepted when we consider how absolutely identical the relations of these two structures are to what is perhaps the most characteristic feature in an Echinoderm, i. e.

the ambulacral system. The histological identity is an additional argument, though of less weight, on the same side.

Accepting this homology as proved, the fact that Crinoids possess part of a nerve-sheath in a primitive and unmodified condition is, to my mind, strong reason for viewing them as descended from forms which agreed with the recent Asterids in possessing a complete nerve-sheath (though possibly very unlike Asterids in other respects), and I am, therefore, disposed to regard the antambulacral nervous system of a Crinoid, i. e. the central capsule and axial cords with their branches, as being derived from the antambulacral part of the primitive nerve-sheath, and not as an entirely new set of structures possessed by no other Echinoderms. A certain amount of evidence can be adduced in support of this view. Dr. Carpenter has shown<sup>1</sup> that in an early stage of development of *Antedon* the radials do not enclose the radial cords, but merely form calcareous plates between the cords and the integument, which later on thicken and grow round, and enclose the cords completely.<sup>2</sup> In this early stage the relations of the radial cords are very similar to those of the ambulacral nerves of an adult Ophiurid or Echinid,<sup>3</sup> and as the latter have certainly acquired their adult condition by becoming detached from the epidermis and shifting inwards, so also may the same process be supposed to have occurred in the Crinoid. The subepithelial bands of the Crinoid retain their primitive positions, but the delicate connective-tissue lamella that sometimes separates them from the overlying epithelium in *Antedon rosaceus*, and is a far more evident structure in *Antedon Eschrichtii* and in *Actinometra*, probably represents the earliest stage in the process by which the nerve becomes detached from the epidermis and shifted inwards. Again, the external and internal plexuses of *Echinus*, with their connecting fibres in the substance of the

<sup>1</sup> Carpenter, 'Phil. Trans,' 1868.

<sup>2</sup> That this condition is a primitive one is shown by its occurrence in some of the Palæocrinoids in which the axial cords often lie in grooves, and not in canals in the calcareous plates (Carpenter).

<sup>3</sup> Of course they do not correspond to these.

calcareous test offer us a condition of things in some respects approaching that of the Crinoid.

Concerning the morphology of the central capsule, I feel in much more doubt. Dr. Carpenter's observations lead to the belief that, at any rate in its present form, it is connected with the change from the pedunculate to the free-swimming condition; and it is worthy of notice that the two actions with which it has been found to be specially concerned physiologically, i. e. the movements of swimming and of righting, are ones that the pedunculate form, from the very nature of things, can never exercise.

While, however, this theory of the derivation of the system of the central capsule and axial cords of a Crinoid by concentration from the antambulacral portion of a continuous nerve-sheath render a comparison between the Crinoids and the other Echinoderms possible, it still leaves the gap between the two groups a very wide one. Crinoids are sometimes compared with Asterids or Ophiurids, but they differ from both these groups in a great number of points of fundamental importance.

In the absence of any representatives of ambulacral ossicles, the convoluted character of the alimentary canal, the position of the anus, the permanent communication between the ambulacral system and the coelom, the replacement (functionally if not morphologically) of the madreporic plate by a number of ciliated openings, we have, quite apart from the entirely exceptional features of the nervous system, a list of characters, which could very easily be added to, which mark off the Crinoids as a group widely separate from the other Echinoderms.

When we bear in mind that in a number of these points the Crinoid condition is not only not a primitive one, but a very highly specialised one, the gap becomes wider still.

I do not propose at present to pursue further this point, which has recently been noticed by both the Carpenters, and will close my paper by venturing to call attention to the great importance of supplementing morphological and histological inquiries by direct experimental investigations. In this age of specialisation there is a very real danger of men

confining their attention too exclusively to one side of the problems they attack, to the entire neglect of others, which are not only of equal importance, but which would in many cases yield them far more ready clues.

Comparative physiology is a phrase which has become well-nigh extinct; but it is the name of a very real and very necessary science, which only requires better opportunities for development, such as we hope shortly to see forthcoming in this country, in order to yield results of first-rate importance to morphologists and physiologists alike.

### DESCRIPTION OF PLATE XXXV,

Illustrating Prof. Marshall's Paper on "The Nervous System of *Antedon rosaceus*."

The figures are diagrammatic, and are intended merely to show the position and relations of the nervous system; the other systems being omitted, either wholly or in part. The nervous system is coloured black in all three figures. In Figures 1 and 3, I have borrowed ideas from figures given by Ludwig ('Morphologische Studien'): all the figures are, however, constructed from camera drawings of my own preparations, and the two mentioned will be found to differ in some important points from the corresponding ones of Ludwig.

#### *Alphabetical List of References.*

*a.* Axial cord. *b.* Branches of axial cord. *c.* Branch of axial cord to pinnule. *d.* Central capsule. *e.* Branches from central capsule to cirri. *f.* Chambered organ. *g.* Central plexus. *h.* Subepithelial band (ambulacral nerve). *i.* Ambulacral groove. *k.* Tentacle. *l.* Ambulacral canal. *m.* Subtentacular canal. *n.* Coeliac canal. *o.* Pinnule. *p.* Cirri. *r.* Mouth. *s.* Intestine. *t.* Ciliated openings in body-wall. *u.* Muscle. *C. D.* Centrodorsal plate. *R.* Rosette. *R<sub>1</sub>.* First Radial. *R<sub>2</sub>.* Second Radial. *R<sub>3</sub>.* Third Radial. *Br<sub>1</sub>.* First Brachial. *Br<sub>2</sub>.* Second Brachial.

FIG. 1.—Diagrammatic vertical section through the disc and base of one of the arms of *Antedon rosaceus*, showing the relations of the central capsule, axial cords, and subepithelial bands. The section is interradiar, *i. e.* passes between two pairs of arms on the left side, radial on the right.

FIG. 2.—Transverse section of an arm of *Antedon rosaceus*, passing on the right side through the base of one of the pinnules. The figure is diagrammatic as regards the branches of the axial cord, which are filled in from a considerable number of sections.

FIG. 3.—Diagrammatic plan of the central capsule and its branches in *Antedon rosaceus*.



# The Development of Phryganids, with a Preliminary Note on the Development of *Blatta Germanica*.

By

**William Patten,**  
Of Boston, U.S.A.

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With Plates XXXVIA, XXXVIB, and XXXVIC.

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## I. GENERAL REMARKS.

THE<sup>1</sup> eggs of this insect (*Neophalax concinnus*) were obtained in great quantities from the muddy bottom of a slow running stream in the neighbourhood of Boston, Massachusetts. About 150 eggs are embedded in a spherical, vesiculated, semi-transparent mass of gelatinous substance, which either lies freely on the top of the mud, or is more or less covered by it according to the length of time it has been deposited. Very rarely it is attached to some stick or stone.

The eggs are found in the greatest abundance in water four or five inches deep, and where there are a great many twigs lying on the bottom and projecting above the surface. These twigs would undoubtedly afford an opportunity for the females to crawl below the water and deposit their eggs in the manner described by McLachlan.<sup>2</sup> I<sup>3</sup> have never seen them myself

<sup>1</sup> A part of the following investigations were carried on under the direction of Dr. E. L. Mark, at the Museum of Comparative Zoology at Harvard University, Cambridge, Mass., the remainder under the instruction of Professor R. Leuckart in the Zoological Laboratory at Leipzig.

<sup>2</sup> 'Monographic Revision and Synopsis of the Trichoptera of the European Fauna,' Robert McLachlan, 2 vol., 1874—80.

<sup>3</sup> The following description applies to Phryganids unless otherwise stated.

ovipositing in this manner, although I have watched them a great many times as they flew over the surface of the water in the neighbourhood of the place where the eggs were found in the greatest abundance.

The eggs are probably dropped by the insect as it alights on any of the numerous twigs projecting above the surface, and since they readily sink there is no necessity of the females going beneath the water to oviposit, as is the case with those Phryganids which fasten their eggs to the under side of various water plants.

Rathke, Zaddach, and McLachlan state that Phryganids lay their eggs, arranged in a single layer and enveloped in a transparent, gelatinous substance, on the under side of various water plants, such as *Hydrocharis morsus ranæ*, *Potamogeton*, and *Nymphæ*. It will be observed that *Neophalax* deposits its eggs in an entirely different manner, and in localities where such water plants are not to be found.

As far as could be observed, the only substance of a vegetable nature available to the larvæ for food was the mass of decaying twigs covering the bottom, which had fallen from the thickly overhanging trees. Unfortunately the stomachs of the larvæ were not examined, so I cannot say with certainty what the exact nature of the food is. It is very probable that the more developed larvæ feed principally upon dead vegetable matter, since I have found such twigs fairly bristling with the larvæ, and at the same time showing many traces of having been gnawed by some insect. The young larvæ, however, devour *Vorticellæ* and various other Infusoria with avidity.<sup>1</sup>

The eggs have a strong odour of "musk," which is still perceptible after they had been hardened by heat and preserved in alcohol for several months, and even more so after they have been softened a half hour or more in water.

The seat of this odour is in the gelatinous envelope since it was not noticeable in the eggs, but, on the other hand, was very strong in the gelatine which had been separated from the eggs.

<sup>1</sup> It has long been known that Phryganid larvæ are in general carnivorous; *Neophalax* is thus an apparent exception to this general rule.

It is extremely penetrating and lasting, and cannot be distinguished from that of ordinary musk; it is quickly absorbed by water and retained for several weeks even after the gelatine has been removed.

To my knowledge this is the first instance that has been recorded where any peculiar odour has been detected in eggs or their envelopes. It may possibly serve as an antiseptic to prevent the too speedy putrefaction of the gelatine, or perhaps to attract parasites and infusoria, which latter furnish food for the young larvæ.

There is no reasonable doubt as to the identity of the species that laid the eggs. Both males and females of this insect, which Dr. Haxgen, of Cambridge, Mass., has kindly identified for me as *Neophalax concinnus*, were observed in great numbers just before twilight flying about over the surface of the water; although watched very carefully they were not observed to oviposit. The eggs are probably laid after dark. Gravid females were found containing eggs and a gelatinous secretion identical in odour and appearance to those found in the water.

Eggs in different stages of development vary considerably in size and shape. They are spherical during the formation of the embryonic membrane as well as after the revolution of the embryo; in all other stages previous to revolution they are oval.

As development proceeds the eggs increase in size to a very marked degree, and by the time they are ready to be hatched the diameter has increased by one fourth or one fifth of its original size. Rathke was the first to call attention to this peculiarity; he observed it in the case of *Gryllotalpa* as well as in Phryganids. I have not only been able to confirm his statements in regard to Phryganids, but also to extend them to one of the *Locustina*, i. e. *Ecanthus*, and to *Blatta*. In both the latter cases the increase in size could not be due to the appropriation of material from gelatinous envelopes since there are none. In all cases the increase in size of developing eggs is probably due to the conversion of a large amount of

highly concentrated and compact food yolk, containing fats and oils, into a less dense and more irregularly distributed protoplasm by the absorption of water, oxygen, and nitrogen from the surrounding media. Oxydation is necessary for all vital activity, and nitrogen is necessary for the conversion of fatty substances into protoplasm. A certain amount of moisture is necessary for the development of eggs which are not normally deposited in water, for such eggs will not develop in a dry atmosphere.

The eggs when first deposited are distinguished by the whitish colour and hard, compact appearance of the gelatinous matrix, which slowly swells and becomes very transparent through the action of the water and decay.

Zaddach states that the larvæ feed upon the softened gelatine and the numerous Infusoria which it contains at the time of hatching. As far as the gelatine itself is concerned he is probably in error, since the larvæ on rupturing the chorion leave the jelly as quickly as possible. Moreover, a great many of these empty gelatinous masses were found which did not indicate that the larvæ had fed upon them. I do not doubt, however, that the larvæ find a welcome store of food in the various Infusoria which infest the gelatinous matrix at the time of hatching.

The question at once arises to what use can these envelopes be applied. To be sure they hold the eggs together in one mass, but as regards protection from their enemies there is more danger in this way than if they were scattered irregularly about. The eggs are undoubtedly eaten by other animals, and when collected into aggregates all suffer the same fate, which would not be the case if they were isolated.

In all Phryganids, with the exception of the present species, the gelatine serves to fasten the eggs to various objects of more or less stability. The eggs of *Neophalax*, as well as some species of *Chironomus*, are suspended in a jelly which floats or lies freely in the water. In other species of *Chironomus*, according to my own observations, the eggs are fastened to some stick or stone.



We have already seen that the gelatinous envelope swells on contact with water, thus decreasing the specific gravity which tends to hold the eggs more freely suspended in the water and prevents them from being too deeply buried in the mud and slime which would cut off the necessary supply of oxygen.

It is possible that the gelatine may have some differential effect upon the diffusion of gases held in the water, excluding some and hastening the transmission of others. It may also serve as an attraction to Infusoria which feed upon the decaying mass, and thus furnish a ready supply of food for the young larvæ. But it is certain that in the great variety of instances where a similar covering is present in other animals which deposit their eggs in the water, e.g. Molluscs, Amphibia, Reptiles, &c., that it must have some other function than the ones just mentioned. For in all the cases mentioned above where we have pointed out a real or supposed advantage accruing to one species or another, it is easily seen that to other closely related forms, and were nearly the same conditions were present, there would be no advantage whatever. No one of these advantages is of such general importance in all the different cases where we find the jelly present as to warrant us in supposing that it was primarily present for that purpose, or, at least, that it had its principal function therein.

There is, however, still another function to which this envelope may be applied, and indeed it is one of far greater and more general application than any as yet suggested. It is, namely, that the gelatinous envelopes of certain eggs which are deposited in the water have their chief function in protecting the eggs against the parasitic growth of fungi and Infusoria. I have observed a great many times that, when the gelatine which envelopes the eggs of *Bufo* or *Rana* has been ruptured and exposed the eggs to a more direct contact with the water, they are very quickly covered and destroyed by fungi. It might well be claimed that the same agent which ruptured the envelope might have killed the embryos, and that of course putrefaction and parasitic growths would follow. But this objection can be easily met by the fact that not only the eggs

nearest the rupture begin to decay, but also five or six, or even more, which lie at varying distances from the rupture, show more or less decay and parasitic growth in proportion as they are near to or far from the break in the gelatine. It is not difficult to find molluscs' eggs (*Limnaeus*) where a similar rupture has occurred, and the subsequent growth of fungi has taken place.

In the case of *Æcanthus*, which deposits its eggs in holes bored in the small branches of various shrubs, if the eggs are removed artificially and kept in a damp place they become quickly covered with such a thick growth of fungi as to prevent further development by shutting off the necessary supply of oxygen.

## II. PREPARATION OF THE EGGS.

Heat alone was found to be the simplest and best means of hardening the eggs and larvæ, which are placed alive in cold water that is very gradually raised to about 60° R.; the proper time to stop the rise in temperature is when they become hard and white. They are allowed to stand till cool, and then removed to 20 per cent. alcohol, which is increased by 10 per cent. once or twice a day until it has reached the required strength, or about 96 per cent. This method has been used with the best success for insect larvæ whose flexible, but impervious, chitinous skins will not allow reagents to penetrate when used in the ordinary way. The great advantage of this method is that it coagulates the tissues without contracting them; the expansion of the air contained in the body prevents the shrivelling of the larva which results when it is treated with cold reagents.

The eggs and larvæ were also heated in Kleinenberg's picro-sulphuric acid solution, one third the normal strength, but with far less satisfactory results.

Since writing the above I have preserved a considerable number of insects' eggs. When picro-sulphuric acid was used and made to penetrate the chorion by means of heat, it was

found almost impossible to remove it again. Eggs of a species of water-beetle—which I believe to be *Hydrophilus*—that were preserved in this way have now been in strong alcohol (96 per cent.) for six months, and they still have a very perceptible yellow colour, due to the picric acid which still remains, and makes it very difficult to stain them with hæmatoxylin. A number of experiments have also been made in the use of corrosive sublimate, but with very unsatisfactory results; the eggs were rendered very brittle, and good staining, which is the chief difficulty in the study of insect eggs, was made impossible. Insect eggs seem to have the power to withstand in a great degree the penetration of staining fluids, which is probably due to the compact tissues, rendered much more so by the use of sublimate, since it causes a considerable shrinkage. At all events, if the eggs are killed by a prolonged and gentle heat, and then hardened in alcohol, no difficulty will be found in staining them, and the histological characters are preserved in as much perfection as by any other method at present at our command.

Bobretzky was the first to use heat in the preparation of eggs for sectioning, but he used it principally as a means to increase the penetration of the chromic acid, a reagent which adds materially to the difficulty of staining them.

The small size of the eggs made it impracticable to remove the chorion, the presence of which proved a serious obstacle to the penetration of staining fluids. Only alcoholic stains would penetrate at all, and of these Kleinenberg's hæmatoxylin gave by far the best results. Partial and varied success was obtained from saffranine dissolved in 90 per cent. alcohol, and Grenacher's alcoholic borax carmine. Saffronine gave better results than the carmine, since it never failed to stain, whereas the latter did.<sup>1</sup>

For the early stages of the egg a light red stain like cochineal

<sup>1</sup> Later experiments, with a 70 per cent. solution of cochineal, have given very good results indeed; and in cases where it is difficult for the fluid to penetrate, this stain produces the required result far better and more rapidly than all others.

or saffronine is the best, since the sections can be cut a little thicker, and still be transparent enough to show the cell structure. This proved of great advantage, since in the early stages the sections, if cut too thin, were liable to crumble on account of the abundance of yolk. When the eggs were stained with hæmatoxylin they were allowed to remain in the staining fluid for five or six days, and were then decolorised very gradually in an extremely weak solution of alcohol and hydrochloric acid—one drop of strong acid to about twenty grammes of alcohol—in which they are allowed to remain several days, and then transferred to pure alcohol, which is changed once or twice until the object has regained its violet colour. Out of a cluster containing about one hundred and twenty-five eggs, only one half or even less could be properly stained. Some were stained only on the outside, although appearing as dark as the rest. By the process of decolourising, such eggs were easily detected and picked out. Some eggs stained more rapidly than others; by leaving them five or six days in the fluid, such as could be at all affected were over-stained. When placed in the solution of hydrochloric acid, all were decolourised alike. The eggs were next transferred successively into absolute alcohol, clove oil, and paraffine, remaining several hours in each.

In treating subsequently the eggs of *Blatta* some changes were made which proved of great advantage. Benzole was used in the place of clove oil; it rendered the eggs transparent almost instantly, and since it is more fluid than clove oil, there is much less danger of the embryo being shrivelled, while at the same time the yolk is not made so hard and brittle. After remaining in benzole for half an hour, the eggs are changed to a saturated solution of benzole and paraffine, which, when allowed to evaporate slowly, gives a perfectly transparent and tolerably hard paraffine. They are next transferred to melted paraffine, which at once replaces the benzole expelled by the heat. Thus there is a great saving of time, since clove oil is not thoroughly replaced by paraffine in less than five or six hours, while with benzole one half-hour is



sufficient. When it was necessary to cut sections of the larvæ, it was found of the utmost importance to transfer them from absolute alcohol to benzole, and then into a solution of benzole and paraffine in the manner described above. The larvæ invariably collapse when placed in clove oil or turpentine. When they are to be mounted in toto the same principal can be applied by mounting them in balsam dissolved in benzol; the solution which can be used thin at first may then be allowed to evaporate slowly until the balsam is of the required density.

On account of the small size of the eggs it was necessary to cut very thin sections. For this purpose the hardest paraffine was required; the melting point of that used was fifty-eight degrees centigrade ( $58^{\circ}$  C.). To get good sections the embedding material must be perfectly clean, hard, and free from bubbles; these conditions can be obtained only by the utmost care in manipulation and in the arrangement of details. Certain advantages are gained by attention to the form of the blocks of paraffine in which the objects are embedded; they should be in the shape of a triangle, the base, which is the shortest side, being just wide enough to hold the object, and the altitude about five or six times the length of the base. The apex of the triangle is directed towards the edge of the knife, which, if placed at an angle of  $10^{\circ}$  or  $12^{\circ}$  to the direction in which it slides, will reach the apex of the triangle first, and cut a closely rolled shaving of paraffine. The section of the object, since it lies in the last portion of the section cut, will occupy the outside roll; it is obvious that, if the triangle was long enough, the roll of paraffine would be so large that a section of the egg would not cover more than one half the circumference. The whole roll may now be placed on the slide, with the section of the object down. When the slide is warmed, the melted paraffine spreads the sections, and thus the trouble of unrolling is avoided.

If in cutting the sections the edge of the knife is placed at right angles to the direction of motion, the sections will not roll, but at the same time they cannot be cut so thin.

Occasionally it is necessary to partly unroll the sections. In this case care should be taken not to use too much clove oil in softening the shellac, or the sections will not adhere firmly enough to allow them to be unrolled. The shellac is also more adhesive if put on the slide just before using. With attention to these details, a roll of paraffine will adhere firmly to the slide, and then may be easily unrolled by means of a fine-pointed needle. When all the sections have thus been placed in rows upon the slide, it is warmed, melting the paraffine, and allowing the sections to lie smoothly on the surface. A very important point is not to warm the slide too quickly, for if there are any sections which do not lie directly on the surface, the paraffine which holds them together will melt and let them fall to pieces before they have had a chance to fasten themselves to the shellac. Hence the slide should be warmed very gradually, and the sections made so flexible that they fall of their own weight into place without the paraffine being actually melted. Moreover, if the slides are heated too much, the shellac and paraffine will unite to form a whitish film, which often destroys the value of the preparation. After cooling the paraffine is dissolved in turpentine or benzole, and the sections mounted in Canada balsam. By this method one hundred sections have been cut from an egg which was one hundredth of an inch in diameter, or, in other words, a nearly complete series of sections each of which was only a little more than one four hundredth of a millimeter thick.

### III. HISTORICAL.

In spite of the innumerable works which have been published concerning the anatomy and habits of insects in general, very little notice has been taken of their embryology. It is remarkable that while our present insufficient knowledge has pointed out such a diversity in the development of closely related forms, that so little attention should have been given to the development of the different groups. The Phryganids, however, have received their proportionate share of attention, since already we have six more or less fragmentary observations concerning

their embryology. One of the earliest references was published by Rathke in Müller's 'Arch.,' 1844. In a footnote (p. 27) he called attention to the increase in the size of the eggs during development. In the posthumous papers of the same author, published by Hagen in the 'Stettiner Entomological Zeitung' for 1861, mention is made of an apparatus for rupturing the egg membranes at the time of hatching. Independently of Zaddach, and, indeed, previous to him, Rathke had also observed the revolution of the Phryganid embryo in the egg, although his notes were not published until several years after the appearance of Zaddach's work. He also described the general external appearance of the eggs, and their occurrence on the under side of the leaves of water plants, *Hydrocaris morsus ranæ*, *Nymphæ*, and *Potamogeton*, &c., as well as their arrangement in concentric circles one layer deep. The eggs, one hundred or more in a single cluster, had two membranes, a thick outer chorion, slightly tessellated, and an inner very thin vitelline membrane; the latter was present when the eggs were first laid, but in subsequent stages was not quite so obvious.

A faint "Keimhaut" was formed containing nuclei. Development was completed in three weeks. Four long and spirally-coiled Malpighian vessels arose behind the stomach. No gills were observed when the larva was first hatched, but thread-like flexible structures appeared later which Rathke supposed to be gills. There was no trace of abdominal appendages.

By far the most important work on the development of "Phryganids" that has yet appeared is the memoir published by Zaddach in 1854. I will therefore give an outline of the more important conclusions reached by this author. The eggs are found in single layered masses on the under side of the leaves of water plants, and are from .009 inch to .011 of an inch in diameter. There are two membranes, an outer, smooth, transparent chorion, and an inner, thin, vitelline membrane. Shortly after oviposition clear spots appear on the surface of the yolk, which gradually increase in number and finally become confluent, forming a transparent outer layer; this, in

his opinion, is a viscid fluid formed by the dissolution of the yolk ; when it has covered the latter uniformly there appears in it isolated white spots—the germ nuclei. These increase in number, and finally cover the yolk with a continuous cell layer. It is claimed here, in accordance with the ideas prevalent at that time, that the nuclei arise first, and that later a cell membrane is formed around them. As soon as the blastoderm is formed its cells are concentrated towards one side, thus leaving the opposite side of the yolk covered by a thin layer, which subsequently ruptures and allows the yolk to come in contact with the vitelline membrane. The germinal band which results from these changes occupies two thirds or three fourths of the circumference of the egg.

The second period, including the metamorphoses of the germinal band and the development of the appendages, begins with two important changes in the former. 1. Its separation into two layers. 2. The formation of two germinal folds or “*Keimwülste*” in the inner layer. The two layers are formed by delamination in the head, and gradually extend to the posterior end of the body. During the formation of these two layers the germinal band not only becomes much thinner in the mid-longitudinal line, but on the side towards the yolk a deep furrow is formed by which the germinal band is partially divided into two lateral ridges, the “*Keimwülste*” thus at an early period indicating the bilateral type and furnishing foundation for the development of the segments and appendages. The inner layer, after giving origin to the nervous system and appendages, forms the muscles of the body walls, and hence is called the “*Muskelblatt*.” The outer layer forms the external covering of the sides and dorsum, the subsequently hardened skin of the embryo, and hence is called the “*Hautblatt*.”<sup>1</sup>

The procephalic lobes are formed by the thickening of a pair of lateral outgrowths from the “*Hautblatt*” in the head region. After their formation and that of the oral invagination the “*Hautblatt*” ruptures in the middle line throughout nearly the

<sup>1</sup> The “*Hautblatt*,” without doubt, corresponds to the “*amnion*” and *serosa* together.



whole length of the original germinal bands. In some cases the rupture is continuous along a transverse line in the head region, and thus a kind of frontal lappet of the "Hautblatt" is left on that portion of the yolk which limits the anterior end of the head. This lappet encloses the so-called head cavity which lies between the procephalic lobes; when, however, in such cases the posterior end of the embryo has grown long enough to reach the head, it fuses with this lappet and thus obliterates the difference between this and the more normally produced condition.

The appendages are evidently developed from the germinal ridges, and push the edges of the ruptured "Hautblatt" away from the middle line, which now exhibits renewed activity along its lateral margin where it remains continuous with the "Muskelblatt." This activity is manifest in a thickening of the "Hautblatt," due to rapid cell-formation which progresses from the lateral to the median edges, and also in the upward growth of this layer which thus envelops the yolk.

The mandibles first arise and then appear simultaneously behind them the five following segments. Although the antennæ appear to develop from the procephalic lobes they are believed to arise from a distinct segment of their own in front of the mandibles, and to correspond to the second pair of antennæ in Crustacea, whereas the antennæ of the adult insect are claimed to be homologous with the first pair of Crustacean antennæ.

The primitive germ band includes hardly more than the future head and thorax. It increases in length by means of new cell formations, which result in the production of an abdomen by the elongation of which the end of the tail comes to lie next to the mandibles. The abdomen consists of ten segments.

The author concludes from the way in which the anal stylets are developed that they are true appendages.

The third period extends from the time of revolution until that of hatching. Four important changes occur in this stage. 1. The conversion of the loose cells into a compact granular tissue from which are formed the different organs. 2. The

closing of the "Scheitel Platte" to form the brain case and the growth of the "Hautblatt" around the yolk, by means of which the body of the embryo becomes enclosed. 3. The gradual contraction of the germinal folds, by means of which the embryo, hitherto encircling the yolk, now comes to lie with its back turned outward, and, 4, the formation of the mesenteron and all other vegetative organs.

Just before revolution the "vitelline membrane" ruptures and collects on the exposed portion of the yolk between the head and tail where it forms a kind of rosette. The author is in doubt whether it forms a part of the dorsal wall or is absorbed by the yolk. The time of the rupture of the vitelline membrane varies, and may even be deferred until after the revolution of the embryo. Sometimes the membrane is absent altogether. The under lip is a ridge-like elevation, which is formed from the mandibular and two maxillary segments with the second maxillæ as a palpus.

After revolution, which is caused by the contraction of the ventral side of the embryo, the latter becomes spirally rolled in the egg, and retains this position until hatching.

The wall of the stomach is formed first on the ventral side and thence grows dorsally.

The eye first appears as six pigment spots. In some cases the lenses to the compound eye could be distinguished.

A sharp-pointed organ found on the forehead serves to rupture the egg membranes. The development covered a period of from eighteen to twenty days.

Previous to Zaddach, Jeder had made some very scanty observations on the nervous system and the eye.

In 1871 Kowalevsky confirmed Zaddach's observations on the membranes, which, after rupturing, form a kind of rosette on the exposed surface of the yolk between the head and tail of the embryo. These observations were made by Wagner, and communicated by him to Kowalevsky.

Brandt, in 1878, referring to the formation of a blastoderm in Phryganids described a clear outer layer in which nuclei subsequently appeared. The origin of the cells was not

observed, but in harmony with his theory he believes that they are direct descendants of the germinal vesicle.

Dohrn, in 1876, also noted the rupture of the membranes and the formation of a dorsal organ. He says that it finally disappears, but does not state in what manner.

#### IV. DESCRIPTIVE.

Stage 1.—In this stage will be included an account of the egg from the time it is deposited until the formation of blastoderm.

The youngest eggs observed were usually ovate in outline, and often more or less irregular from mutual pressure. The viscid, greenish yellow yolk completely fills the chorion and contains two kinds of rounded elements; those of one kind are albuminoid in nature, staining deeply in all the common reagents; those of the second kind are colourless, much more refractive than the first, and exceedingly variable in size. They are dissolved in the processes preceding imbedding, so that in sections their former existence is indicated only by the presence of circular cavities in the yolk; from this it is to be inferred that they are oil globules.

Within ten or or twelve hours after oviposition, the time varying with the temperature, a clear space makes its appearance at the surface of the egg, and gradually increases until it has attained the breadth of the future blastoderm. In this layer, which has been called the "blastema,"<sup>1</sup> the protoplasm has, under ordinary conditions, a very homogeneous appearance, with occasionally lighter, less refractive spots which appear like vacuoles, but in which, when observed more closely and under slight pressure of a cover-glass, or especially when treated with a very little acetic acid, faintly marked nuclei

<sup>1</sup> There is not the slightest doubt that in many insects a structure precedes the true blastoderm, presenting many well-defined characteristics which distinguish it from the blastoderm; hence it requires a special name. I can see no objection to retaining the name "blastodermic blastema," or simply "blastema," originally proposed by Weisman, even though his view that the nuclei of the blastoderm arise in this layer spontaneously is incorrect.

make their appearance in greater or less numbers, according to the more or less advanced stage of the blastema.

From the study of the living egg one might readily infer that the nuclei arise as free formations in the protoplasm, since in no case could they be observed to ascend from the depths of the yolk into the "blastema;" and, indeed, Weisman claimed this to be the case in the *Diptera*, although later he withdrew his statement. Strictly defined, and in the sense that we use the term, the "blastema" is a thin, nucleated layer of protoplasm covering the whole outer surface of the yolk, and not divided into distinct cells. It may be remarked here, that it is not impossible, or even improbable, that a "blastema" may occur in some instances without nuclei, although at present this has not been observed to occur. How such a condition could be brought about will appear later.

The true structure, however, of the "blastema" can only be learned by the study of carefully prepared sections, and we shall then be convinced that the above definition is correct. Such sections through the long axis of the egg in the early stages of the "blastema" are represented in Pl. XXXVIb, figs. 1, 2, 3. Here one may observe: (1) spherical masses of yolk more or less modified by contact with each other; (2) yolk masses whose outlines are so modified as to enclose circular cavities; (3) amœboid cells, each containing a large and sharply stained nucleus; (4) an irregular network of protoplasm whose amœboid filaments are usually connected with the cells just described; and (5) a thin layer of protoplasm at the periphery, containing a few nuclei, and continuous with the protoplasmic network in the interior of the egg. Sections of a stage slightly later than the one just described show (fig. 2) that the blastemic layer has increased in thickness, and is supplied with a considerable number of regularly arranged nuclei. Both the protoplasmic network and the cells observed in the previous stage have disappeared from the yolk. In the next stage observed (fig. 3) the protoplasmic layer has become more sharply defined, the nuclei being more numerous and regularly arranged than in the previous stage. The surface of



the protoplasm has an undulating outline, showing the first indications of division of the syncytium into distinct cells. At this period also no nuclei are to be seen in the yolk. The explanation of these facts is very simple. The nuclei first seen in the yolk have arisen by the division of the germinal vesicle, and together with the protoplasm scattered through the yolk are migrating towards the surface. In the later stages it will be observed that all the germ cells have reached the surface, forming a syncytium, the blastema, which speedily develops into the blastoderm.

There is considerable variation in the relative amount of protoplasm and the number of nuclei which may be found at the surface at a given time. Very often the protoplasmic layer would attain a thickness equal to that of the future blastoderm, and yet contain but a very few scattered nuclei; while, on the other hand a large number of nuclei could be seen at the surface, where there was hardly enough protoplasm to form a continuous layer. Hence it is not only possible, but also probable, that a blastema might in some cases occur in which there were no nuclei. For if in one species, as in *Neophalax*, we can have a blastema containing a very few or a great many nuclei, it is not improbable that in other insects the variation may be carried a step farther and produce a layer of protoplasm in which there are no nuclei. Such a modification would be of extremely slight morphological value, since it is a condition which is dependent on the fact that sometimes the protoplasm reaches the surface first, and sometimes the nuclei. We have, however, seen that such variations can actually exist in one species. Still, the fact remains to be proved by means of sections that a blastema actually does exist without nuclei.

The yolk<sup>1</sup> presents two varieties; in one the globules are perfectly homogeneous, and stain deeply; in the other they

<sup>1</sup> The greater part of this section on the structure of the yolk applies chiefly to *Blatta*, on which the most complete observations were made. From what was seen in Phryganids, I should think that the same description would apply to that group also. In Phryganids the yolk is not so compact, and the amount of protoplasm is proportionately much larger.

stain less deeply, and are composed of a loose, apparently granular mass, which, however, on closer inspection, reveals the presence of so many irregular vacuoles as to reduce the yolk substance to a fine network. Such yolk masses are most abundant near the surface. They are probably merely varieties of the first kind, brought about by its gradual absorption to supply food for the rapidly developing germ cells. It is an important characteristic of the yolk that it is not transformed into protoplasm simply by the solution of its outer boundary, as a small block of ice would be dissolved in warm water; on the other hand, the yolk balls do not decrease perceptibly in size, but simply become less dense, and finally are changed into a more or less fine network of deutoplasm, the walls of the yolk ball still retaining its sharply defined outline. It is possible that these cavities in the deutoplasm may have been filled with an oily or fatty substance, which had been dissolved by the reagents used in the preparation of the egg. That a great deal of soluble fatty substance is present in the yolk from the first we have already seen; but from the configuration of the solid yolk around spaces once filled with fat we can see that a great deal of the substance in question was formed of isolated globules. Since in the very early stages of development the yolk has that homogeneous appearance first described, and the granular yolk only appears after the germinal cells have begun to increase rapidly, the only explanation available is that this transformation of the yolk is brought about either by the gradual absorption of the yolk at many different points in each globule, or the yolk may be first gradually transformed into small fat globules which are then converted into protoplasm, and absorbed by the amœboid cells. If the eggs had been treated with alcohol and benzole, this fatty substance would have been dissolved, producing the net-like or granular yolk balls just described. It is indeed not easy to decide by which of these processes the yolk is absorbed, since we have no facts at hand which point more plainly towards one conclusion than another.

If the yolk was decomposed by direct contact with the

cell protoplasm, we would expect to find a close coincidence existing between the distribution of the germ-cells and the granular yolk. This is, however, not the case, for if we examine sections of the eggs of *Blatta* cut through the longitudinal dorso-ventral plane we shall find, when there are only a few cells in the centre of the egg, that the deutoplasm has already become granular at the periphery; later, when the cells are distributed regularly throughout the egg, that a very thick layer of granular deutoplasm has been formed on the ventral side directly under the place of the future ventral plate. Hence it would appear that the yolk transformation goes on independently of the germ cells. The fact that the granular yolk is found for the most part at the periphery indicates that the decomposition was produced by a kind of breathing process. This supposition is rendered improbable by the fact that the ventral surface of the egg, which has the greatest amount of granular yolk, has the least air, being turned towards the centre of the cocoon in which the eggs are laid. Since the transformation of the yolk is not governed entirely by the supply of air, although we cannot deny that a certain amount is a necessary condition, and also, since it is not produced by the direct action of the living germ-cells, we must look to some other agent for the conditions requisite for bringing about such a transformation. Since then, the yolk is metamorphosed on the ventral side, anticipating, as it were, the advent of the germ-band, and supplying beforehand the necessary amount of ready food for the subsequent rapid development of cells at this point, it may be influenced by the same conditions which determine the subsequent location of the germinal band at that place.

Stage 2.—The second stage is of comparatively short duration, and is marked by only two important features—the formation of the blastoderm and its differentiation into the ventral plate.

The blastoderm is formed by the simultaneous aggregation of a definite mass of protoplasm around each nucleus of the blastema. These masses quickly acquire cell walls, thus forming

the first true cells of the embryo, for hitherto we have had a "syncytium." By mutual pressure the outer faces of the cells become highly convex. The nuclei are large, and each contains a small, sharply-stained nucleolus. When seen from the surface they appear as represented in Pl. XXXVIA, fig. 1; in section as in Pl. XXXVIB, fig. 4.

The blastoderm at first surrounds the yolk as a single layer of cells of uniform thickness, which soon become long and columnar at one pole of the egg, and correspondingly thin and epithelial-like at the opposite pole (Pl. XXXVIB, fig. 5). The thin portion of the blastoderm becomes the "serosa," and the thickened part is the ventral plate. Zaddach states that the blastoderm ruptures at the dorsal pole, where it becomes so thin. This, however, is a mistake, as a long series of sections at this stage, and even much later, shows the complete continuity of the original blastoderm at all points of the egg.

Stage III.—The period covered by this stage extends from the time of the formation of the ventral plate up to the completion of the embryonic membranes. It includes the process of the gastrulation, the origin of the endoderm, and the mesoderm, as well as the formation of the amnion and serosa.

The embryonic membranes are first seen as faint elevations of the "ventral plate," which in optical section appear like a thick layer of columnar cells (Pl. XXXVIA, figs. 3 and 4). In actual section they appear like simple folds of the blastoderm (Pl. XXXVIB, figs. 8 and 9), which are raised on all sides of the embryonic area, and gradually increase in extent until they finally meet nearly over the middle of the ventral plate. For the sake of convenience in description one may recognise four separate folds—a head fold, tail fold, and two lateral folds—although, as we have already indicated, they form a continuous, somewhat circular, elevation. Pl. XXXVIB, fig. 8, represents a longitudinal section through the ventral plate, showing the head fold at *am''* and the tail fold at *am'*; the latter arises first, and grows more rapidly, often covering more than two thirds of the ventral plate before the lateral folds meet in the central line. This condition is not easy to observe in the living egg, but



a very fair idea of it can be obtained by comparing Pl. XXXVI<sub>A</sub> figs. 3 and 4.

The ventral plate has a characteristic dome-like shape, so that its outline is strongly arched, both in cross and longitudinal sections.

At this stage the ventral plate is not much longer than it is broad. The cells composing it are columnar, with well-defined, rounded outer ends; the inner ends, on the contrary, are faintly marked, and send delicate, pseudopodial filaments into the yolk. The protoplasm is very faintly granular; it is also vacuolated, and especially in the vicinity of the nuclei. Bobretzky<sup>1</sup> speaks of vacuoles in the endoderm cells of *Oniscus*, which possibly may be of the same nature.

During this stage of development certain nuclei present very striking and characteristic appearances. Although most abundant during this stage they may be observed to occur with varying frequency up to the time of the revolution of the embryo. The most noticeable but less frequent form is that which has a very deeply-stained margin, and contains a highly-refractive, non-stainable substance, in the centre of which may occasionally be observed fine granules, scattered about irregularly, or so concentrated as to form a kind of central axis (Pl. XXXVI<sub>C</sub>, fig. 33). Another variety is seen in the nuclei represented in Pl. XXXVI<sub>C</sub>, fig. 35): they are sharply limited, and contain very darkly stained filaments, which at first sight appear like granules, but by careful focusing with a highly-magnifying lens one can distinguish a continuous network of fibres, which in optical section look like granules; they are in marked contrast with the highly refractive and non-stainable substance which constitute the remainder of the nucleus.<sup>2</sup> A third variety is seen in the great majority of cells which constitute the embryo; they stain deeply and uni-

<sup>1</sup> N. Bobretzky, "Zur Embryologie des *Oniscus murarius*," 'Zeit. für wiss. Zool.,' Bd. xxiv, 1874.

<sup>2</sup> Compare H. Flemming, 'Zellsubstanz, Kern und Zelltheilung,' Leipzig, 1882.

formly, excepting the minute, faint granules, and the small, darkly-stained nucleolus.

A spindle metamorphosis was not observed in the embryo, although the nuclei often contained two nucleoli, and were probably undergoing rapid division. The small size of the nuclei, however, rendered them unfavorable for the study of such phenomena.

These different kinds of nuclei are distributed irregularly throughout the ventral plate, and are so constant in their characters that it is difficult to find stages which might be considered transitional. The first two varieties possibly represent stages preliminary to division. It should be noticed that the ordinary nucleus in a state of division presents no other peculiarity than the presence of two nucleoli.

It will be seen on examining Pl. XXXVI<sub>B</sub>, figs. 8, 9, and 10, that the nuclei of the ventral plate are uniformly located at the inner or deep ends of the cells, while those of the amnion are at the outer ends, thus maintaining in both layers the same morphological position. From this we can infer that the amnion is simply a fold of the thickened ventral plate, and the serosa merely the slightly modified blastoderm covering the remainder of the egg, and which gradually extends over the ventral plate in a fold, whose lips finally meet and fuse, thus producing two completely closed sacs.

It can be seen, in Pl. XXXVI<sub>B</sub>, fig. 8, when the folds are first forming, that the inner limb (*am*) has the same thickness as the ventral plate itself, and also that the nuclei have the same morphological position, while, on the other hand, the outer limb (*sr*) is very thin, being simply a continuation of the blastoderm. These relations are still perceptible in much later stages (figs. 9 and 10), even when the membranes are completely formed (fig. 10). Thus, according to this view, the real edge of the ventral plate is not where the amnion joins the ventral plate, but where the amnion is continuous with the serosa.

At present I cannot claim any special morphological value for these distinctions, since all the parts in question are directly

descended from the blastoderm; neither is it possible as yet to say how far such distinctions hold good in other forms. Judging, however, from Kowalevsky's<sup>1</sup> figures I should think that the amnion was a derivative of the ventral plate. It is the same in *Porthesia* figured by Bobretzky,<sup>2</sup> although the difference is not so well marked here as in *Hydrophilus*.

We have already seen that the fusion of the amniotic folds with each other produces two completely closed sacs, one within the other (Pl. XXXVI B, figs. 10—13). The outer sac formed by the serosa, which is not fully represented in the figures, completely encloses the yolk. The second sac, the amnion, is also completely closed, its dorsal wall being much thickened to form what we shall presently describe as the "germinal band."

We have seen that the ventral plate in the restricted sense in which we use the term is simply an oval thickening of the blastoderm covering the ventral side of the egg. During, however, the formation of the amnion and serosa this thickened area takes a more definite shape, increasing in length and decreasing in breadth, thus giving rise to a slipper-shaped thickening, which we shall now call the "germinal band." The head of the future embryo arises from the "heel" of this band, which at this place expands into two plates, one on each side, in order to form the procephalic lobes. Zaddach's statement that they arise as thickenings of the "Hautblatt" is erroneous; for, as far as I am able to make out, he confounded the amnion, or possibly both amnion and serosa, with the ectoderm.

Up to this time the germinal band consists simply of a single layer of similar cells; it, however, continues to increase in length, until at the end of this period it extends over two thirds of the circumference of the egg.

Meantime, however, there have occurred peculiar changes affecting the apparent localisation of the ventral plate and germinal band. The thickening of the blastoderm occurs at one

<sup>1</sup> A. Kowalevsky, "Embryologische Studium an Würmern und Arthropoden," 'Men. Ac. imp. St. Petersburg,' Ser. vii, vol. xvi, 1871.

<sup>2</sup> Bobretzky, "Ueber der Bildung des Blastoderms u. Keimblätter bei den Insecten," 'Zeit. für wiss. Zool.,' 1878, vol. xxxi, pp. 195—215.

pole of the egg, and may be said to lie parallel with its short axis (Pl. XXXVI<sub>B</sub>, fig. 5, and Pl. XXXVI<sub>A</sub>, fig. 2). During the formation of the embryonic membranes the egg becomes spherical; and still later, just before the appendages have appeared, it again assumes an oval form, the ventral plate now, however, extending in the direction of the long axis of the egg.

These changes are brought about by an increase in the length of the ventral plate, and its tendency to lie straight in the egg. This is rendered possible by the flexibility of the chorion, which yields to such an extent as to readily allow the transformation of the short diameter into the long one. When the ventral plate has attained about one half its ultimate length the egg assumes a circular form, owing to the equilibrium existing between the extensive force of the germ band and the tendency of the egg to retain its oval shape. This circular form, however, is retained but a comparatively short time, for in about twenty-four hours the germinal band has so increased in length as to convert what was the short axis into the long one (compare Pl. XXXVI<sub>A</sub>, figs. 4, 5, and 6); here the egg is seen from the dorsal side, and simply shows the two ends of the germinal band, which has already begun to encroach upon the dorsal surface of the egg.

During the formation of the embryonic membranes there may be observed a median longitudinal infolding of the germinal band (Pl. XXXVI<sub>B</sub>, fig. 9), which commences in the posterior end of the embryo and gradually extends towards the head, almost reaching the point where the mouth appears later; in optical section it has the appearance shown in Pl. XXXVI<sub>A</sub>, fig. 5. It will be seen in the next stage that this fold entirely disappears (Pl. XXXVI<sub>B</sub>, fig. 11), and is not to be confounded with a median fold in the same place that appears later, and which initiates the formation of the nervous system (Pl. XXXVI<sub>B</sub>, fig. 12). The first furrow gives rise to a part of the endoderm and all of the mesoderm, hence we will call it the "gastrula."<sup>1</sup>

<sup>1</sup> Objection has been made to the use of the term "gastrula" in this connection, on the ground that the invaginated cells do not give rise to the endoderm. This objection has in the present instance been met by showing that



The second furrow gives rise to a part of the nervous system, and hereafter we shall call it the "neural furrow."

The gastrula then is quite open at first, but its lips gradually close, so that the only trace of the opening left is that shown by the arrangement of the invaginated cells and their nuclei (Pl. XXXVI<sub>B</sub>, fig. 10). The closing of the gastrula mouth begins at the posterior end of the body, and gradually extends towards the head end. In no case do the infolded cells form a closed tube. In two or three hours the furrow has entirely disappeared.

The cells which are thus permanently cut off from a direct share in forming the outer wall of the body constitute the beginning of the mesoderm. Although the boundaries of these cells are no longer sharply defined, the position of their nuclei shows clearly the relation which they sustain to the rest of the ventral plate, and is suggestive of the stages which have immediately preceded. As seen in Plate XXXVI<sub>B</sub>, fig. 10, these nuclei are already arranged in two rows approximately parallel with the nuclei of the neighbouring ectoderm. With the depression of the dome-shaped ventral plate which occurs at this period, the mesoderm is changed into a single layer of cells covering the under surface of the ventral plate (Pl. XXXVI<sub>B</sub>, fig. 11). This movement, however, may not necessarily imply any marked amœboid properties for the cells at this period; they have increased in numbers, and the shifting incident upon cell division has played an important part in the changes of position that have been observed.

Zaddach's description of the splitting of the ventral plate into a "Hautblatt" and "Muskelblatt" can in no way be compared to the formation of the mesoderm as described above.

at least a part of the endoderm does arise from invaginated cells. But even if this was not so, I cannot see that it would influence our use of the term in this case. For it seems to me that an inherited tendency to produce an invagination, the sole object of which is to cause a differentiation into germ layers, however successful or unsuccessful the attempt may be, should receive the name that was originally applied to the more simple condition, always holding in mind, however, the fact that it is a condition modified by various agents, the nature of which may or may not be known.

What he described as the "Hautblatt" was probably the amnion and serosa combined, and the "Muskelblatt" the ventral plate. The same author has figured an infolding similar to that described as the "gastrula," but which, in his opinion, is simply the secondary result of the formation of two lateral thickenings or germinal ridges out of which the segments and appendages of the body are formed. He states that the ridges result from a lateral contraction, which causes a furrow to appear on the inner surface of the ventral plate, and not by an infolding of the outer surface, as shown in fig. 9, Pl. XXXVIb. It is difficult to decide which of these two infoldings Zaddach had under observation when he wrote his description, but judging from the fact that he ascribed the origin of the nervous system to the germinal ridges on each side of the furrow, I think it probable that what he saw was simply the neural furrow.

The endoderm arises from any point in the blastoderm by DELAMINATION, and this process may continue even after the blastoderm has been converted into the ventral plate.

It will be remembered that no cells were left in the yolk after the formation of the blastoderm. By examining sections of eggs during the formation of the embryonic membranes, amœboid cells, containing large granular nuclei, can be seen in the yolk both in the region of the ventral plate and in that of the serosa; in the latter one may occasionally find cells projecting into the yolk and sending out ray-like arms of protoplasm; very often two cells may be seen close together, one in the serosa, the other just inside the yolk and connected with each other by protoplasmic filaments. Other more or less similar cells can be seen in the immediate vicinity of the ventral plate, especially along the median longitudinal line. Admitting the accuracy of the observation in regard to the entire disappearance of the nuclei from the yolk in the blastoderm stage, there are thus only two possible interpretations for the present condition; either these yolk cells rise autogenously in the yolk and are migrating towards the periphery, or certain cells of the serosa and ventral

plate have undergone division, and one of the products of that division having assumed amœboid characters, is now in the process of freeing itself from the blastoderm preparatory to migrating into the yolk. There can be no doubt as to which of these explanations is correct. There is not the slightest evidence of the free formation of cells within the yolk, and their intimate connection with the serosa and ventral plate points clearly to the cells of these structures as the source of their origin. These so-called yolk or endoderm cells, which later become somewhat modified in size and appearance, are gradually distributed through all parts of the yolk and ultimately form the epithelial lining to the mesenteron.

Stage 4.—This period extends from the completion of the embryonic membranes up to the appearance of the appendages; it is marked by the segmentation of the mesoderm.

The germinal band is now a thick layer of cells, extending over about three fourths of the circumference of the egg, and continuous at its periphery with the amnion, which meantime has become very thin. Surrounding both amnion and germinal band, may be seen the serosa, with its large granular nuclei, from which the amnion can readily be distinguished by the small size of its nuclei. The two cell layers ultimately become very closely united, although never completely fused, since in sections of the embryo just previous to revolution it can readily be seen in many places, especially near the nuclei of the amnion, that they are separate membranes.

The amnion always maintains its continuity with the extremities of the body, so that the amniotic cavity remains completely closed, even up to the time of the rupture of both amnion and serosa. The extreme tenuity of the amnion at this stage is in marked contrast with its appearance when first formed, it being then as thick as the ventral plate.

The invagination which gave rise to the mesoderm and part of the entoderm occurred during the formation of the embryonic membranes, and disappeared when the latter had completely fused with each other, hence no trace of an invagination is to be observed during this stage. It is of great

importance to observe the complete disappearance of the gastrula invagination and the existence of a stage following it where there is no median furrow. The neural invagination, as we shall soon see, is a second and later process, and although it occurs exactly in the place where the gastrula mouth disappeared, it has no connection with it whatever. The definite comprehension of this fact will, I believe, prove quite as important a step towards the comprehension of the phenomena of insect development, as has the similar distinction between primitive groove and medullary furrow in the case of Vertebrates. This distinction between the neural furrow and the gastrula has not heretofore been clearly recognised in insect embryos. Zaddach surely confounded them, and I find no evidence that any observer has called attention to the fact that there are two fundamentally distinct invaginations occurring successively in the middle of the ventral plate.

The mesoderm at the beginning of the present period is unsegmented, and forms a continuous layer of cells beneath the ectoderm; it soon separates along the median longitudinal line, thus forming a pair of lateral mesodermic bands, each of which at the same time divides into segments or somites, which, however, do not contain a lumen or body cavity as described by Hatschek and Kowalevsky, but are simply formed by the apposition of two cell layers, which later split into the splanchnic and somatic mesoderm.

The ventral plate, as seen in the living egg at this time, shows a gently undulating surface, the external evidence of the segmentation of the body, the depressed areas alternating with the mesoblastic somites. The formation of the external depressions and the segmentation of the mesoderm occur at the same time.

In many places in the mesoblastic somites, cells may be seen which present all stages of migration from the mesoderm into the yolk. Some of these mesoderm cells can be distinguished from those surrounding them by having larger and more granular nuclei; others are partly separated from the mesoderm being still united with the latter by a delicate filament of



protoplasm (Pl. XXXVI<sub>B</sub>, figs. 6 and 7), and still others which have completely separated themselves from the mesoderm and lie in the yolk close to the ventral plate; in general appearance all the cells just described are very similar to each other. Although I am not ready to believe without further evidence that yolk cells arise by proliferation from these segmented portions of mesoderm, yet it is necessary to recognise the fact that the appearances just described could be employed as arguments in favour of such a view. A great many cells similar to those represented in Pl. XXXVI<sub>B</sub>, fig. 6, were observed in various stages of separation from the mesoderm. In the upper part of fig. 6, Pl. XXXVI<sub>B</sub>, is represented a cell which is connected with the mesoderm by a very long filament of protoplasm. Such a condition would not be realised in cells approaching the mesoderm, but very likely would be if the cells were separating from the mesoderm; for in the latter case it is very likely that a filament would continue to hold the elements a long time in union. It may be urged, on the other hand, that the absence of any indication of a spindle metamorphosis is, to a certain extent, an objection to the supposition of a proliferation on the part of the mesoderm cells; this objection, however, appears less important when it is remembered that no spindle metamorphosis could be observed in other parts of the embryo where there was undoubtedly rapid cell division.

Stage 5.—The fifth period extends from the segmentation of the mesoderm to the revolution of the embryo in the egg. It is the longest and most complicated period in the development of the insect; at its close nearly all the organs of the body are differentiated.

No important change occurs in the general form of the germinal band up to the time of revolution. The procephalic lobes increase in thickness and at the same time the yolk withdraws from between them, allowing their inner faces to come in contact and unite with each other to form the brain. The germinal band continues to increase in length until its extremities almost touch each other, and even at that late period it is very easy to distinguish in the living egg the points where

each extremity is continuous with the amnion (Pl. XXXVIA, figs. 9 and 10, *am.*<sup>1</sup> and *am.*<sup>2</sup>). After the appearance of the proctodæum the posterior end of the body becomes bent forward on itself, making the first step towards the revolution of the embryo.

Formation of the Appendages.—Shortly after the growth of the ventral plate has brought the two extremities into close proximity with each other, six pairs of gentle undulations appear simultaneously on the anterior half of the body (Pl. XXXVIA, fig. 8). The three posterior pairs, which ultimately become the thoracic appendages, are somewhat longer than the others. The three anterior pairs become respectively the mandibles, and the first and second maxillæ. As development proceeds each of these appendages assumes a characteristic shape and direction of growth. The mandibles (Pl. XXXVIA, fig. 9) become globose, and lie at right angles to the median plane of the body. The first maxillæ diverge very early from their original rounded form, becoming elongated and pointed at their extremities; they are much larger than either the mandibles or the second maxillæ, and maintain their superiority in size until the embryo hatches. The second maxillæ imitate the first maxillæ in shape and direction of growth.

The three thoracic appendages are very similar to each other in size and outline and in their manner of development. When they are about half grown three pairs of rudimentary appendages may be seen in each of the three anterior abdominal segments (Pl. XXXVIA, fig. 11). The thoracic appendages are at first directed towards the median line of the germinal band, being in marked contrast with the rudimentary jaws, which are directed away from the middle line of the germinal band. No further important changes occur either in the direction of growth or in the shape of any of the appendages until just before revolution, when the second maxillæ fuse to form the under lip and the first maxillæ at the same time turn towards the median ventral line and assume their ultimate arrangement around the mouth-opening. The thoracic appendages become very much distorted just before the rupture of the amnion and serosa (Pl. XXXVIA, fig. 14).

The upper lip is formed by a fold in the germinal band just in front of the mouth; its cavity, which is lined with mesoderm, opens directly into the body cavity (Pl. XXXVI<sub>A</sub>, figs. 9, 10, 12, 13, 14, and *f. hd.*).

The antennæ appear as knot-like protuberances on the posterior edges of the procephalic lobes (Pl. XXXVI<sub>A</sub>, fig. 10, &c., *at.*). Here it will be seen that they are very prominent; gradually, however, they become less conspicuous, until in the later stages they are scarcely to be observed at all. When, however, the embryo has emerged from the chorion the antennæ again increase somewhat in size, although still remaining quite small. Zaddach's statement that a second pair, corresponding to the first pair of crustacean antennæ, appears after hatching, the first pair having entirely disappeared, is erroneous.

The nervous system is primarily indicated by a shallow groove, beginning at the anterior end of the body, and extending backwards almost to the tip of the germinal band, terminating at the place where the proctodæum is to be formed. As development proceeds, the cells on each side of the groove divide and give rise to faintly marked nuclei, each of which contains a small deeply stained nucleolus (Pl. XXXVI<sub>B</sub>, figs. 12 and 13). By the increase in numbers of these cells, a continuous cord is formed on each side of the median furrow. This condition, however, does not last long, for, on the dorsal side of these cords, deeply stained granular nuclei appear (Pl. XXXVI<sub>C</sub>, fig. 21, *x*), which finally increase to such an extent as to almost take the place of the faintly marked nuclei (Pl. XXXVI<sub>C</sub>, fig. 23). These granular neural cells at first form a single layer directly under the neural cords. I at first thought them to be transformations of the nuclei above them, but certain indications render it possible that they were formed by division of the ectoderm cells on each side of the neural cords (Pl. XXXVI<sub>C</sub>, fig. 21, *y*), for in stages slightly earlier than that represented in the figure, these cells were observed only at the point marked *y*, and appeared to grow inwards towards the median line. This point, however, I was not able to determine with certainty.

Soon after their formation the neural cords become transversely divided into ganglia. Meantime the median furrow has increased in depth, forming an invaginated portion of the ectoderm which unites transversely each pair of ganglia. This median portion of the nervous system gradually disappears. Whether it actually forms the cross commissures, as claimed by Hatschek, is still an open question. While my own observations have not confirmed those of Hatschek, still, nothing was found that actually militates against his theory.

If we examine longitudinal sections of the embryo after the middle cord has disappeared (Pl. XXXVIc, fig. 37), we shall observe two granular spots or commissures on the dorsal side of each ganglion. These spots increase in extent, and at length fuse with corresponding tracts in the opposite half of the ganglion, thus uniting the lateral halves of each pair of ganglia by two cross commissures (Pl. XXXVIc, figs. 37 and 38). When the embryo is ready to hatch these double commissures disappear; whether they appear again in the adults of this insect I am not able to say. Such commissures, however, are present in the adult crab, and certain insects—*Locusta*—according to Leydig; but whether they are of the same nature as those seen in embryo Phryganids, or whether they are secondary modifications of these embryonic conditions, is a question that we are not in a position as yet to answer. At all events, I have not been able to find a description of such a condition in either insect or crustacean embryos.

Coincident with the ectodermic invagination joining the lateral halves of each ganglion, transverse invaginations are formed between the successive ganglia themselves (Pl. XXXVIc, fig. 37). Here also Hatschek asserts that the infolded cells give rise to the long commissures. When such an invagination has once been proved to exist, as it undoubtedly does, one does not have to go far for an explanation; in fact, that offered by Hatschek is very plausible, and the only one at hand. But this is one of the cases where it is extremely easy to find a probable explanation, but very difficult indeed to prove that it is really the case. In other words, we lack the positive



proof that the invaginated ectoderm cells give rise either to the long or the short commissures.

Before the formation of the brain by the union of the thickened procephalic lobes, a deep fold is formed on the lateral surface of each lobe, which results in the formation of a thin blade of ectoderm, which imperfectly divides the brain into an anterior and a posterior portion. This fold is seen in surface view in Pl. XXXVIA, figs. 11 and 12, and a section of the same in Pl. XXXVIc, fig. 41. Although not indicated in fig. 41, since the stage is too late, there is an actual infolding of the ectoderm produced, which, according to Tichomerof ("Ueber die Entwickl. des Seidenwürms," 'Zool. Anzeiger,' ii, Jahr. No. 20 (Preliminary Notice)), produces a part of the chitinous internal support for the brain, while, on the other hand, Hatschek claims that it forms a part of the ganglionic cells of the brain. As an objection to Tichomerof's view, it can be urged that this fold is fundamentally different from the thickened rod-like ectodermic ingrowths which undoubtedly form a part of the internal skull (Pl. XXXVIB, fig. 16, *a*, and fig. 15, *d*). These unfoldings, however, are exactly like those between the post-oral ganglia, and hence lead to the conclusion that the brain is formed of two pairs of coalesced ganglia, from the posterior pair of which the antennæ are developed, the anterior pair producing no appendages. The principal objection to this view is that instead of having a single transverse fold as in the post-oral segments, we have in reality two separate, lateral infoldings, one on each side. The lateral position, however, is easily explained, since the procephalic lobes, originally strictly ventral in position, have been bent in a dorsal direction, giving them a lateral position as related to the germinal band. The fact, however, that there are two folds is a real difficulty, and can only be explained by assuming that the fold which originally extended along the whole ventral face of the brain has disappeared in the middle line, thus leaving two separate and lateral, although originally continuous, infoldings. We have, however, no facts to warrant such a supposition. If the brain in reality is made by the fusion of two pairs of ganglia, then

the antennæ which develop from the second pair of ganglia are homologous with the second pair of crustacean antennæ; structures corresponding to the first pair of crustacean antennæ are not developed in insects. Our knowledge of the development of the brain in Crustacea is as yet so imperfect as to forbid any comparison between its development and that of insects. I hope, however, to be able to make some investigation on this subject from a comparative point of view.

A cross section of the brain, perpendicular to the anterior surface of the head, discloses a pair of solid rod-like ingrowths of the ectoderm, which form a part of the endocranial support for the brain (Pl. XXXVIb, fig. 16). There is no infolding at the surface to mark their points of origin. A second pair is shown in sections dorsal and parallel to that just described; they originate from the posterior wall, and project forward and inward (Pl. XXXVIb, fig. 15).

Bridging a broad and deep invagination of ectoderm in the middle of the anterior face of the head, there is developed a low conical structure with a sharp apex. It is the same organ described by Zaddach as an apparatus used in rupturing the chorion at the time of hatching (Pl. XXXVIb, fig. 15, *c*).

A very remarkable club-shaped organ lies just beneath the structure just described; at its lower end it is somewhat narrowed and is attached to the anterior surface of the brain; from this point it projects upward into the narrow space between the brain and its chitinous wall, and there terminates freely. It is bounded on each side by an infolded portion of the ectoderm, which forms a part of the endocranium. When seen in section (Pl. XXXVIb, fig. 15, *b*) it appears like a tube whose wall is thrown into folds, the centre being occupied by a clearer and less granular substance. These two peculiar organs, which appear to stand in intimate relation with one another, I regard as the rudiments of the simple eye, the so-called "egg-rupturing apparatus" being the modified lens (?) whilst the club-shaped organ beneath is a special nerve centre, corresponding to the ring-like structures seen, according to Leydig, in the brain of ants. If, according

to Rathke and Zaddach, and Brandt among recent authors, this pointed organ serves to rupture the egg membrane—which I doubt very much, the only evidence of that purpose being in its apparent adaptability—its chief function probably does not lie in that alone, being merely a secondary adaptation of its primary function as a sense organ.

The compound eyes appear, just before the revolution of the embryo on the surface of the procephalic lobes, as round refractive areas, in which subsequently six dark red pigment spots appear (Pl. XXXVI<sub>A</sub>, figs. 13—16). A section through the eye at this period shows a thickened area of ectoderm, which ultimately becomes connected with the brain (Pl. XXXVI<sub>B</sub>, figs. 15 and 19).

Tracheæ are formed in all the post-oral segments, with the exception of the last two or three abdominal. The tracheal invaginations are less conspicuous in the abdominal segments than elsewhere; they are formed by an invagination of the ectoderm close to the nervous ganglia (Pl. XXXVI<sub>B</sub>, fig. 20, *t*). In the thorax and head the invaginations occur on the outer or dorsal sides of the appendages, the tracheæ thus formed lose their connection with the exterior, and, increasing in length in an antero-posterior direction, fuse with each other and form common tracheal trunks one on each side of the body.

The spinning glands are formed by a pair of ectodermic invaginations on the ventral side of the embryo, between the base of the second maxillæ and the nervous cord. They increase rapidly in length until they are nearly two thirds as long as the embryo, and when the second maxillæ fuse they also unite to form a common duct, which opens at the end of the upper lip.

The salivary glands are formed by invagination of the ectoderm on the inner sides of the mandibles, in the same manner as are the spinning glands. They are short tubes reaching back behind the second maxillæ.

The mesoderm in the preceding stage had separated into segments, the lateral extremities of which were formed of two irregular layers of cells; no definite body cavity had appeared.

During the present stage the mesoderm increases in extent, and at the same time the cells composing it become less definitely and compactly arranged. The yolk, on account of its absorption by the growing embryo, becomes reduced in amount, and gradually recedes from the ventral plate. Into the cavity thus formed amœboid mesoderm cells migrate, and give rise to the blood-corpuscles (Pl. XXXVIb, fig. 20). As the yolk continues to withdraw from the ventral plate the mesoderm follows it by a rapid growth along its lateral margins, which, when they come in contact with the yolk, are reflected backwards and inwards towards the median line, thus forming, on either side of the body, a mesodermic layer, which bounds the yolk on the ventral side.

By continued growth the margins finally meet each other.

The mesoblastic wall, however, does not entirely separate the yolk from the body cavity, for it is segmented in such a way as to produce bands of mesoderm extending entirely across the ventral side of the yolk, alternating with other bands, which are interrupted in the middle line, thus allowing free passage from the yolk into the body cavity. A section through one of these interrupted bands is shown in Pl. XXXVIb, fig. 20, where one or two yolk-cells are represented in the act of migrating from the yolk into the body cavity.

In the meantime the lips of the mesoblastic folds have progressed in an opposite direction, as shown by arrow No. 1 (Pl. XXXVIb, fig. 20), giving rise to two layers of mesoderm, an inner and an outer. The inner layer or splanchnic mesoderm extends over and encloses the yolk, with its muscular layer before the yolk or endoderm cells have formed the epithelial lining of the stomach; the outer layer or somatic mesoderm becomes closely united to the ectoderm, with which it extends dorsally to form the outer wall of the sides and dorsum. Before the folds have met in the middle line of the dorsum the amnion and serosa rupture, so that the further consideration of the changes in these layers will be deferred until the next stage.

The endoderm cells increase very rapidly during this stage,



both in number and size. Their nuclei have become enormous, and it is only occasionally that one can distinguish protoplasm surrounding them. In size and general appearance they closely resemble granular yolk masses, and if their transition had not been traced step by step one would not believe that they were cells. Before the splanchnic mesoderm has completely separated the yolk from the body cavity on the ventral side some of the yolk-cells migrate into the body cavity, where they arrange themselves irregularly along the sides of the body wall. They are at once distinguished from the surrounding mesoderm cells by their great size and amœboid protoplasmic arms. At first the cells are arranged singly and indefinitely along the outer wall of the body; but later, by increase in numbers, they become arranged in distinct groups, which will be described more definitely in the next stage.

Exceedingly large vesicular cells, and probably of the same origin as those just described, are seen during this stage on the ventral side of the œsophagus, where it comes in contact with the yolk (Pl. XXXVIc, fig. 22, *en. c.*, and Pl. XXXVIc, fig. 36, *a*).

When the embryo is ready to hatch, these cells have disappeared, although their exact fate I was not able to determine with certainty.

In the latter part of this period the yolk-cells begin to arrange themselves upon the walls of the yolk-sac. During this process they become less distinct and gradually reduced in numbers, owing to the migration of some of them into the body cavity. In the anterior part of the yolk-sac it was extremely difficult to observe yolk-cells in the act of forming the epithelial lining of the stomach, since the cells were both so indistinct and few in number. On the other hand, it was comparatively easy to observe this in the posterior part of the yolk-sac, since the cells were both distinct and numerous; the epithelial lining of the stomach is probably formed first in the neighbourhood of the proctodæum, and then extends forwards to the stomodæum. It is certain, however, that the yolk-cells do not form a continuous sac until some time AFTER the formation of the mesodermic musculature of the yolk-sac. This

would account for the statement of Tichomerof, that the mesoderm gives rise to the epithelial lining of the mesenteron.

The Malpighian vessels (Pl. XXXVI B, fig. 17) arise as THREE SEPARATE PAIRS of hollow outgrowths from the inner blind end of the proctodæum, one dorsal, one lateral, and one ventral; the latter pair appears first, and is somewhat larger than the others; from this pair also a fourth pair originates close to its points of origin. Further increase in the number of vessels is not produced by fresh evaginations of the proctodæum, but by budding from the first three pairs of Malpighian vessels.

Stage 6.—The time from the rupture of the embryonic membranes to that of hatching constitutes a sixth period. The most important changes which occur are—(1) the rupture of the embryonic membranes resulting in the formation of the dorsal organ; (2) the revolution of the embryo within the chorion; and (3) the formation of the heart.

At the beginning of this period the embryo assumes a peculiar shape, due to the fact that the abdomen has become bent back upon itself, a part of its ventral surface being now concave instead of convex. When the embryo has reached the condition represented in Pl. XXXVI A, fig. 14, the amnion and serosa, which have become confluent or, at least, closely united, rupture along the line of confluence, the free edges of the membranes being reflected back upon the sides and dorsum; the rapidity with which they move towards the dorsal region indicating their elasticity and the high state of tension to which they have been subjected. They now concentrate on the dorsal portion of the yolk to form a rosette-shaped dorsal organ, which gradually disappears from the surface (Pl. XXXVI A, fig. 15). A complete series of sections at successive stages (Pl. XXXVI c, figs. 39, 40, 42) shows beyond a doubt that the dorsal organ sinks into the yolk and is absorbed. In the stages immediately following the retraction of the membranes the nuclei are very sharply defined, and are embedded in a clear mass of protoplasm in which is soon formed a tubular invagination nearly perpendicular to the dorsal surface of the

yolk. A longitudinal section of this invagination is to be seen in Pl. XXXVIc, fig. 39. The whole dorsal organ now sinks into the yolk, the nuclei become less distinct, and by the confluence of its sides the invagination disappears (fig. 40). Still later no trace of the nuclei can be detected; they have gradually faded, and finally disappeared altogether from the protoplasmic mass without shifting their position. The protoplasmic matrix in which the nuclei were embedded does not break up into fragments, but disappears *en masse* some time after the nuclei.

After the rupture of the membranes, and during the changes which take place in the dorsal organ, the abdomen of the embryo becomes folded more and more towards the ventral side, until the curvature of the embryo becomes completely reversed (compare Pl. XXXVIa, figs. 12 and 16).

After the dorsal organ has passed into the yolk, the lips of the uprising mesoderm, which on either side unite the splanchnic and somatic layers, gradually envelop the yolk, and finally meet each other along the middle line of the dorsum. By the fusion of these two mesodermic lips a solid cord of cells is formed occupying the median longitudinal line of the back indicating the first foundation of the heart. A cross section of the heart at this period is represented in Pl. XXXVIc, fig. 32, from which it will be observed that the cells have fused into a common mass of protoplasm in which the nuclei are irregularly arranged.

Transverse sections through the embryo just before hatching show that this cord of cells has become hollow; the cavity thus formed is the lumen of the dorsal blood-vessel.<sup>1</sup> I am unable to say whether any of the nuclei of this cord are isolated and fall into the lumen during the histological differentiation of its walls which are formed by the production of cells from the syncytial mass. These cells give rise to successive muscular rings, each of which is composed of two transversely striped muscle cells, each cell forming one of the lateral halves

<sup>1</sup> It was probably this similarity which led Weismann to think that the heart of the fly was formed by the hollowing of a solid muscular cord.

of the ring and containing near its centre a single nucleus (Pl. XXXVIc, fig. 30).

Just before the embryo hatches all the yolk-cells have migrated to the periphery of the yolk, where they arrange themselves to form the epithelial lining of the mesenteron. About the same time the thin membranes at the blind ends of the stomodæum and proctodæum rupture, and their cavities become continuous with that of the mesenteron.

The remaining organs of the body which were already formed during the preceding stages do not undergo any important changes during this period.

#### THE LARVA.

The larva, which resembles that of *Mystacides* figured by Zaddach, immediately after its escape from the egg forms a case of fine sand and small fragments of vegetable matter.

In larvæ several weeks old no gills were present unless the vesicular appendages between the anal stylets can be considered as such. When, however, the larva is about three sixteenths of an inch long a pair of filamentous tracheal gills bud from the lateral walls of each segment of the abdomen.

Sections of very young larvæ show the complete formation of all the larval organs. The body cavity is broken up into irregular spaces bounded by thin membranes, on the walls of which there may be seen a number of large cells whose protoplasmic filaments extend across the cavity, and either unite with the opposite wall or with similar filaments from other cells (Pl. XXXVIc, fig. 29). These cells are the same that we have already observed in the preceding stages attached to the body wall, and which had migrated into the body cavity from the yolk-sac.

The salivary glands have already assumed the peculiar and characteristic appearance that they have in the adult. A cross section through one of them (Pl. XXXVIc, figs. 25 and 26) shows in the centre an exceedingly small duct surrounded by a zone of radiating filaments; outside the latter, and constituting



the remainder of the section, there is a wide zone of a finely granular substance in which one may observe scattered nuclei.

#### SUMMARY.

The most important results at which I have arrived may be summarized as follows:

In the earliest stages observed there were already a number of germ-cells in the yolk, together with an irregular network of protoplasm. ALL THE NUCLEI and protoplasmic network migrate to the surface and form a syncytium or "blastema," which, by the segregation of definite masses of protoplasm around each nucleus, is converted into the "blastoderm," which then becomes thickened at one pole to form the "ventral plate."

From any point of the ventral plate, or serosa, cells arise by budding and migrating into the yolk and form the so-called "yolk-cells," the greater part of which ultimately form the epithelial lining of the mesenteron, and hence are to be regarded as true entoderm cells which have arisen by DELAMINATION from the ectoderm. After the segmentation of the mesoderm certain cells were observed in stages of separation from the mesodermic segments, which probably migrate into the yolk, and then cannot be distinguished from the ordinary yolk-cells.

During the formation of the embryonic membranes there is formed a median longitudinal invagination, resulting in the production of a continuous plate of mesoderm. The groove formed by this invagination quickly disappears, and then follows a stage in which the ventral plate has no median depression. Very soon, however, a SECOND DEPRESSION originates along the same median line, which initiates the formation of the nervous system. The latter is formed by the differentiation of a pair of lateral cords arising from the division of the ectoderm cells lying on either side of the neural furrow, and by the addition of a median infolded portion of the ectoderm, which may possibly form the cross commissures. There are also transverse infoldings of the ectoderm between the contiguous segments.

The mesoderm separates early along the median line, forming a pair of lateral bands, which soon break up into segments, in each of which arises an imperfectly closed space, the body cavity. The splanchnic mesoderm grows under the yolk in alternating, continuous, and interrupted bands. Through the openings in the interrupted bands some of the yolk-cells pass into the body cavity. Before the embryonic membranes rupture the yolk-cells have already begun to form the lining of the posterior part of the mesenteron.

Tracheæ are formed in all the post-oral segments, except the last two or three segments of the abdomen.

The spinning and salivary glands are formed by special ectodermic invagination on the inner side of the second maxillæ and the mandibles respectively.

The Malpighian vessels arise as six separate evaginations of the blind end of the proctodæum.

The embryonic membranes, after rupturing, retreat towards the back, where they form the dorsal organ, which at one stage is simply a vertical tube-like invagination that quickly disappears, and the whole mass of cells which constitute it gradually sinks into the yolk and is absorbed.

A solid cord of cells is formed in the median dorsal region by the fusion of the edges of the mesodermic folds. The cord becomes hollow and forms the heart.

#### CONCLUDING REMARKS.

No group in the animal kingdom shows such a wonderful morphological and physiological diversity as the Tracheata, and this tendency to vary is as strikingly manifested in the development of the individual as in the adults themselves. Even closely related groups present the widest variations in their fundamental processes of development. Hence the few facts obtained from the development of one species do not warrant wide generalizations.

The variation in the amount of yolk always affords a convenient and often a very plausible explanation for these modified processes of development, but it will not account for everything,

and in many cases we must look beyond this for an adequate explanation of the facts.

The yolk doubtless plays a very important part in modifying the segmentation of the egg; the amount is so great that if it were intimately combined with the protoplasm it would prove a very serious obstacle to segmentation, and hence to accelerate development it is from the first entirely separated from it.

We may conceive that the germinal vesicle is surrounded by an irregular mass of protoplasm, scattered widely through the yolk. When the nucleus divides, only a small portion of the protoplasm collects around the resulting nuclei, the remaining portion being irregularly distributed throughout the yolk. After a number of germinal cells have been formed, exactly the same conditions would exist that we have first observed (Pl. XXXVIb, fig. 1).

Both the germ-cells and outlying protoplasm are subjected to some centrifugal influence which propels them towards the surface. This might give rise to different results, varying with the amount and condition of the yolk. It would be a very slight and unimportant modification of the more ordinary course of events to have the network of protoplasm reach the periphery first, and thus form a superficial protoplasmic layer before the less mobile nuclei could migrate through the yolk and reach the surface. The impediment offered to the nuclei by the yolk would easily account for the presence in some insects (*Musca*, *Chironomus*, &c.) of what Weismann named the *Keimhautblastem*, whereas the absence or diminution of this impediment would in other cases (e.g. *Aphis*, &c.) allow the nuclei to arrive at the surface simultaneously with the accumulation of protoplasm. The Phryganids offer a condition which is intermediate between these extremes.

One of the most remarkable results due to the presence of so much yolk is the production of three distinct stages in the segmentation of the egg; first, the germinal vesicle with its protoplasm divides and produces a number of cells which unite at the surface to form a syncytium; second, the syncytium quickly divides into a number of cells with cell walls; and third,

long after the germinal band is formed, and the appendages have appeared, the yolk itself undergoes a cleavage of greater or less extent. The attempt has been made to show that the cleavage of the yolk is due to some centripetal force of the yolk-cells; if this was the only cause we should have one of two results dependent upon the relative strength of this centripetal force and the inertia of the yolk particles. If, on the one hand, the force were strong enough to overcome the inertia of the yolk particles, yolk-balls would be formed around each nucleus; this indeed is often the case, as, for instance, in *Lina* and *Hydrophilus*, and certain *Phryganids*. On the other hand, if this force were not strong enough to overcome the inertia no cleavage would take place. In other words, provided the force of each nucleus is the same, and they are regularly distributed, either a complete cleavage into a number of segments equal to the number of nuclei will take place, or no cleavage at all will result. For instance, the condition shown in *Neophalax* would never be realised by such a disposition of forces; for in this case the yolk is simply divided into two or three segments in which there are a great many nuclei. However, in *Mystacides* (*Zadach*) there are no more yolk-cells present than in *Neophalax*, yet there is a complete segmentation of the yolk. There is no doubt that a division of the yolk by increasing the exposed surface would greatly enhance its transformation and absorption by the embryo, and for that reason, other things being equal, we might expect to find a definite relation existing between the extent of segmentation and the duration of the development. This, however, does not appear to be the case, for in *Ecanthus* (one of the *Locustina*) the yolk is completely segmented, and the eggs which are laid early in the fall do not hatch until the next summer; while in *Blatta germanica* the yolk is very slightly segmented, and the development lasts but a few weeks. At present, therefore, a great many more facts are necessary before it will be possible to discuss the question with any probability of arriving at a definite conclusion.

It seems very probable that in all insects at least a large portion of the mesoderm arises by an infolding along the



median line of the ventral plate. This has been observed in all cases where the development has been carefully studied. Kowalevsky was the first to call attention to it, and his observations have been confirmed by Graber, 1877, and the Hertwigs, 1881. In the Phryganids, all the mesoderm arises from this invagination. Tichomeroff, 1879, claims that the mesoderm is not formed by an invagination, but by budding from the cells of the blastoderm and primitive ventral plate. Since the gastrula stage is of such short duration, it was probably not included in the eggs which he sectioned, and the cells observed to bud from the ventral plate and blastoderm are in reality endoderm, instead of mesoderm cells.

Graber had already claimed, in 1871, that some of the endoderm cells were formed from the blastoderm. Balfour, however, discredits his statements and believes that the phenomena observed by Graber were in reality due to the passage of yolk-cells into the blastoderm. It is very probable, however, that Graber is right in his conclusions, and that he observed the same process that we have described for Phryganids. In this case, however, we have more conclusive evidence, for ALL THE GERM CELLS having migrated to the surface, it is much easier to observe a passage of cells from the blastoderm into the yolk. In the later stages, where yolk-cells apparently bud from the mesodermic segments, it is not possible to state with the same confidence the direction in which the cells are migrating. The facts, however, seem to indicate, as we have already stated, that some of the yolk-cells are formed at this late period by budding from the mesodermic segments. This does not appear so improbable when we reflect that the segmentation of the yolk, a process which ordinarily precedes the formation of the endoderm, occurs also at this period.

The dorsal organ, or something equivalent to it, is found in all insects whose development has been carefully investigated. Uljanin and Bobretzky have described the dorsal organ of Amphiphods as a saddle-shaped area of cells attached to the vitelline membrane, and extending over the dorsal region of the body; the whole layer becomes disconnected from the

embryo except by a central umbilicus. It finally, however, becomes completely separated, and in *Orchestia* sinks into the yolk.

As Bobretzky has hinted, it is very probable that this saddle-shaped area of cells is homologous with the embryonic membranes of insects. If we imagine, for instance, that the ventral plate of *Oniscus* had extended nearly around the egg and then the embryonic membranes formed just as in insects (without, however, extending completely over the ventral surface of embryo), and if the membranes thus formed should fuse with the vitelline membrane, we should then have an organ which would be like the dorsal organ of *Oniscus*, and at the same time homologous with the embryonic membranes of insects. If, to carry the comparison still farther, the dorsal plate of cells should separate from the vitelline membrane, and, contracting, sink into the yolk, we should have still further grounds for comparison. In fact these conditions are realised in embryo Amphipods and Isopods, and show that the saddle-shaped plate of cells in *Oniscus*, &c., undergoes a series of changes, parallel with those of the embryonic membranes and dorsal organ of insects. A careful distinction, however, should be made between the dorsal organ of Insecta, and that of the Phyllopods and Nauplii of Decapods, &c., all of which have been considered by Dohrn as homologous structures. As a matter of fact, however, the only points they have in common is that all are located in the neighbourhood of the dorsum and that very little is known about them.

It will be observed from the description already given of the germinal band with its gastrular and neural invaginations that a wonderful analogy at least, if not homology, exists between these structures and the corresponding ones in Vertebrates, and especially birds. For instance, in both bird and insect the germinal band has the same slipper-shaped outline. The primitive streak of birds and the gastrula-groove in insects, both of which are homologous structures, begin at the posterior end of the germinal band and extend forwards. Likewise the medullary furrow in both groups arises at the anterior

end of the germinal band, and extends towards the posterior extremity, the only difference being that in insects the medullary or neural furrow is formed in nearly the same place where the gastrula occurred, while in birds the medullary furrow is located somewhat in front of the primitive streak.<sup>1</sup>

Still another point of comparison between the nervous system of Vertebrates and insects is shown by the fact that in both groups a differentiation into two kinds of nerve-cells occurs—an inner layer with granular nuclei, and an outer layer, composed of faintly-marked nuclei. The former layer corresponds to the grey nerve-matter of Vertebrates, and the latter to the white substance. This differentiation in structure indicates the same physiological differentiation of the nervous system in both groups.

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### *Blatta Germanica.*

Since the completion of the paper on *Neophalax*, a few observations have been made upon the development of *Blatta Germanica*, some of which may be mentioned here preliminarily, reserving a more detailed and complete account until the observations on this form have been completed.

In the embryos of *Blatta* as well as in those of most if not all other insects, there appears in each of the segments at a certain time a great number of clear, highly refractive particles that at first might be taken for oil globules, and which have always been regarded as such. On more careful exami-

<sup>1</sup> The gastrula-groove in insects disappears from the anterior end of the germinal band, backwards; while the medullary or neural furrow, which commences somewhat in front of the anterior extremity of the gastrula-groove, is forming in the same direction; before, however, the gastrula-groove has entirely disappeared from the posterior extremity, the medullary furrow has extended to the posterior end, where it opens into the gastrula, both becoming continuous and forming a NEURENTERIC CANAL, just as in birds and other Vertebrates with meroblastic ova.

nation, however, it will readily be seen that this supposition is incorrect. A number of tests have been made in order to ascertain the nature of these bodies, and the results show that they are some salts of uric acid. That they are not of a fatty nature is indicated by the fact that treatment of the embryos with hot benzole, chloroform, or clove oil has not the slightest effect upon the bodies in question. Further examination with a highly magnifying power shows that they consist of small spheres of an extremely refractive substance, from the centre of which dark lines radiate in an irregular manner, producing the same appearance seen in the crystals of urea from the Malpighian vessels. It was this similarity which first suggested the true nature of these bodies, and further tests confirmed this view, for after heating an embryo with nitric acid upon a glass slide, and then adding a little ammonia, the characteristic red colour of Murexid was formed. A still further test was used by dissolving the granules in dilute caustic potash, and then precipitating the urea by adding acetic acid, although this method did not give such definite results as the first.

A rather striking variation was found in the first and second maxillæ of *Blatta*, which were formed respectively of two and three branches, the second maxillæ thus attaining the typical trichotomous structure of the Crustacean appendages.

At first a number of abdominal appendages are developed, which, however, quickly disappear again with the exception of the first pair, which further develops into pear-shaped structures attached to the abdomen by a stem that increases in length and finally changes into a very fine duct leading into a small cavity in the expanded distal extremity, which owes its size to the development of extremely high ectoderm cells. No mesoderm enters into the construction of this peculiar organ, which in the later stages of development disappears entirely. This organ is undoubtedly a special development of the first abdominal appendages, yet its function is extremely problematical. Rathke observed what is very likely a similar organ in the embryos of *Gryllotalpa* and which he regarded as a rudimentary gill, but the remarkably thick ectodermic walls of this structure makes



this supposition improbable. It is more likely that it had some sensory (?) function on account of the peculiar structure and development of the ectoderm cells, while at the same time the longitudinal duct leading into a cavity into which the large cells could pour a secretion points to the supposition that it is possibly of a glandular nature.

In *Blatta* the heart is formed by the fusion of mesodermic folds similar to those in Phryganids; instead, however, of forming a solid cord the heart is from the first hollow. The mesodermic folds pulsate regularly long before they have united to form the heart.

#### NOTE.

Since the completion of this paper on *Blatta* and Phryganids the work of Dr. A. Korotneff on the development of the heart in *Gryllotalpa* has appeared ('Zool. Anzeiger,' December 24th, 1883), which contains several points on the origin of the heart and blood-corpuscles resembling what I have found in *Blatta*. The blood-corpuscles both in *Blatta* and Phryganids are formed from free mesoderm cells, which, however, do not arise in the median line of the embryo, as this author has indicated, but from the apex of the angle made by the united splanchnic and somatic mesoderm.

The condition shown in *Gryllotalpa*, in which the semicircular projections on the uprising mesodermic folds are formed before the folds have hardly begun to grow towards the back, is not attained in *Blatta* until the folds have very nearly united with each other in the median dorsal line. Moreover, a thin layer of mesoderm always closes in the yolk dorsally before the mesoderm folds unite, thus preventing a communication between the blood system and the yolk-sac, as Tichomerof has described in *Lepidoptera*.

I cannot believe that the condition represented by Korotneff is possible. In the first place it is improbable, on theoretical grounds, that a blood sinus should be formed simply between the vitelline membrane and the yolk, as he described. In fact,

I do not believe such an extended space as he described exists at all, unless produced by shrinkage due to reagents. The yolk is separated from the vitelline membrane by the upgrowing mesoderm folds, and the only space formed is just before the folds unite to form the heart, and this space is simply the cavity of the heart.

The primitive blood sinus is not the space between the lips of the mesoderm folds, however large or small this may be, for this space is not in communication with the body cavity at all, and even when the mesodermic folds first begin to beat the space contains no blood-corpuscles. The primitive blood sinus, then, is the space between the somatic and splanchnic mesoderm, divided into a number of smaller and irregular sinuses by meshes of connective tissue, some cells of which, in the earlier stages, become free and form the blood-corpuscles. By the pulsation of the mesodermic folds, long before a special heart is formed, a circulation through the irregular sinuses of the body cavity is brought about, like the circulation in many of the lower Worms.

By the union of the mesodermic folds a definite heart is formed, from whose walls, in the earlier stages, a number of blood-corpuscles are produced in addition to those formed in the true body cavity. I hope to produce absolute proof of this statement, accompanied by drawings of cells in different stages of separation from the dorsal inner wall of the heart, when all my observations on *Blatta* are completed and published. These facts add the evidence of embryology to that of comparative anatomy, to show that the heart of Worms and Arthropods is a comparatively new structure, which was preceded by a circulation produced by contraction of the muscular walls of the body cavity.

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EXPLANATION OF PLATES XXXVIA, XXXVIB,  
XXXVIC.

Illustrating Mr. W. Patten's paper on "The Development of Phryganids, with a Preliminary Note on the Development of *Blatta Germanica*."

*List of Reference Letters.*

*am.* Amnion. *an.* Anus. *at.* Antenna. *b. cv.* Body-cavity. *bd.* Blastoderm. *bl.* Blastema. *br.* Brain. *cm.* Cross commissures. *d. o.* Dorsal organ. *ec.* Ectoderm. *en.* Endoderm. *f. hd.* Fore head. *ht.* Heart. *l. cm.* Long commissures. *md.* Mandible. *mp. v.* Malpighian vessels. *ms.* Mesoderm. *mx.*<sup>1</sup> and *mx.*<sup>2</sup> Maxillæ 1 and 2. *n. f.* Neural furrow. *œ.* Œsophagus. *p.*<sup>1 2 3</sup> Thoracic appendages. *pc. l.* Procephalic lobes. *pr.* Proctodæum. *sl. g.* Salivary glands. *sp. g.* Spinning glands. *sr.* Serosa. *tr.* Trochæ. *v. p.* Ventral plate. *y. c.* Yolk-cells. *gn.* Ganglion.

## PLATE XXXVIA.

FIG. 1.—Lateral view of the egg in the blastoderm stage.

FIG. 2.—Same view of the egg four hours later, showing the thickening of the blastoderm to form the ventral plate.

FIG. 3.—Lateral view of the ventral plate during the formation of the embryonic membranes. It is shown as a semi-transparent mass of cells; the headfold at *am.*, and tailfold at *a.*

FIG. 4.—Same embryo one hour later, the head and tailfold have approached each other preparatory to uniting.

FIG. 5.—Dorsal view of the anterior end of the embryo. The amnion and germinal band are seen in optical section. *gst.*, gastrula invagination.

FIG. 6.—Same view of the embryo, after the gastrula invagination has disappeared. The embryo has increased in length, so that the ventral plate extends the whole length of the egg. At *a* is a segmentation furrow dividing the yolk into halves.

FIG. 7.—Lateral view of the embryo, seen from the left side. None of the appendages have as yet appeared.

FIG. 8.—The same embryo twenty-four hours later, showing the first appearance of the appendages.

FIG. 9.—Same embryo, two days later than that represented in Fig. 7. The ventral plate is seen to be continuous with the amnion at both extremities of the body, *am.*<sup>1</sup> and *am.*<sup>2</sup>

FIG. 10.—Lateral view of the embryo in about the middle of the fifth stage.

FIG. 11.—Same embryo two days later. At  $p.^{4, 5, 6}$  may be seen the rudimentary abdominal appendages;  $a. st.$ , anal stylets in optical section.

FIG. 12.—Lateral view of an embryo about twelve days old. The tip of the abdomen is bent back as far as the eighth abdominal segment.

FIG. 13.—Lateral view of the embryo in the latter part of the fifth stage. The tip of the abdomen has reached the sixth abdominal segment.

FIG. 14.—Embryo, just before the rupture of the embryonic membranes;  $fz.$ , bodies, which in *Blatta* we have shown to be salts of urea, and which are undoubtedly of the same nature here.

FIG. 15.—Lateral view of the right side. Embryo in the last stages of revolution, the membranes have ruptured and collected on the back to form the dorsal organ,  $d. o.$

FIG. 16.—Lateral view of the embryo from the right side, after the completion of revolution.

#### PLATE XXXVIB.

FIGS. 1, 2, and 3.—Three successive stages in the formation of the "blastema."

FIG. 4.—Longitudinal section through the egg in blastoderm stage.

FIG. 5.—A similar section during the formation of the ventral plate.  $a.$  Yolk-cells in the act of separating from the serosa.

FIG. 6.—Section through a mesodermic segment, showing two cells in the act of separating from the mesoderm and passing into the yolk.

FIG. 7.—Longitudinal section of the ventral plate, showing another cell in the act of separating from the mesoderm.

FIG. 8.—Longitudinal section through the ventral plate, showing the formation of the embryonic membranes. At  $y. c.$  and  $x.$  are two cells in different stages of separation from the ventral plate and serosa.

FIG. 9.—Cross section of ventral plate in head region, showing the gastrular invagination at  $gst.$

FIG. 10.—Section similar to that in Fig. 9, only in a later stage. The embryonic membranes have united over the median line of the ventral plate. The gastrular invagination has disappeared, leaving a group of median cells behind.

FIG. 11.—Cross section in thoracic region before the appendages have appeared. The mesoderm has spread out into almost a single layer of cells,  $ms.$  The gastrular groove has entirely disappeared.

FIG. 12.—Cross section through the ventral plate during the early stages of the nervous system. A few nuclei have appeared at the deep ends of the ectoderm cells, on either side of the neural furrow. The mesoderm has separated from the median line and is thickened at the outer margins.



FIG. 13.—Cross section of the ventral plate in a later stage. Beneath the median furrow is a group of cells uniting the lateral cords.

FIG. 14.—Cross section of the abdomen during the stage represented in Pl. XXXVIA, fig. 12, showing a section of the proctodæum and the Malpighian vessels.

FIGS. 15, 16, 19 are successive sections of the head in a plane perpendicular to the front wall of the cranium. Fig. 16*d* shows a pair of ectodermic rods forming a part of the endocranium. Fig. 15 is a section dorsal to the one just described; *c*, the so-called egg-rupturing organ, whose function, however, is still very doubtful, and which may represent the simple eye of Insects; *b*, a structure, probably of a ganglionic nature, connected with the simple eye; ectodermic ingrowths are developed on either side of these organs. Fig. 19, a section dorsal to that in Fig. 15, showing at *a* the ectodermic thickenings to form the eye; *en. c.*, a group of vesicular endoderm cells on the ventral side of the œsophagus.

FIG. 17.—Cross section of the blind end of the proctodæum, showing the three separate pairs of invaginations to form the Malpighian vessels.

FIG. 18.—Cross section of the folded abdomen in the region marked *an. st.*, Pl. XXXVIA, fig. 12. *a* is the median infolded portion of the ectoderm uniting the lateral cords; *a. st.*, anal stylets.

FIG. 20.—Cross section of first abdominal segment. *en. c.*, yolk-cells migrating into the body cavity; *so. m.*, somatic mesoderm; *sp. m.*, splanchnic mesoderm. The arrows indicate the directions in which the mesodermic folds are growing.

#### PLATE XXXVIC.

FIG. 21.—Cross section through the first maxillæ and procephalic lobes. In the deep surface of the lateral cords dark granular cells appear, which finally replace the more lightly stained ones. The stage corresponds to that shown in Pl. XXXVIA, fig. 10.

FIG. 22.—Longitudinal section of embryo corresponding to Pl. XXXVIA, fig. 12.

FIG. 23.—Cross section through the mandibular segment of an embryo corresponding to Pl. XXXVIA, fig. 12.

FIG. 24.—Cross section through abdomen of larva. *a*, segmentally arranged thickenings of the hypodermis; *y*, one of the wandering endoderm cells.

FIG. 25.—Cross section of salivary gland of larva.

FIG. 26.—Longitudinal section of a part of the same gland.

FIG. 27.—Muscle cells of the larva.

FIG. 29.—Section of one of the large cavities in the body of the larva. *d*, amœboid endoderm cells attached to its wall.

FIG. 30.—Cross section of the larva, showing the fully formed heart, *hb.*, and the wall of the mesenteron.

FIG. 31.—Part of a section through a larva. *a*, segmental thickening of the hypodermis opposite the tracheal trunk, *tr*.

FIG. 32.—Section through dorsal region of an advanced embryo, showing thickened cord of cells from which the heart is to be formed. *e. n.* yolk-cells, attaching themselves to the wall of the yolk-sac to form the mesenteron.

FIGS. 33, 34, and 35.—Very highly magnified cells of the ventral plate, showing various kinds of nuclei.

FIG. 36.—Cross section of the embryo through the first pair of maxillæ, showing the tracheal invaginations; at *a* are very large endoderm cells.

FIG. 37.—Sagittal section of the three thoracic ganglia, showing the double cross commissures.

FIG. 38.—A longitudinal section of the same ganglia, showing the longitudinal and double cross commissures.

FIG. 39.—Longitudinal section of the dorsal organ of an embryo shown in Pl. XXXVIa, fig. 15.

FIGS. 40 and 42.—Successive stages in the disappearance of the dorsal organ.

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On the Development of *Halisarca lobularis*  
(O. Schmidt).

By

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Late Fellow of St. John's College, Cambridge, Professor of Geology in the  
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With Plate XXXVII.

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IN the summer of 1883, I spent—thanks to the hospitality which Professor Lacaze Duthiers so generously extends towards foreigners—some weeks in the Zoological Laboratory at Roscoff, Brittany, and while there was permitted to preserve for subsequent examination specimens which were not among the immediate objects of my research at that time. In this way I obtained several examples of both species of *Halisarca*, of which on my return to England I cut numerous sections, and then found that the specimens of *Halisarca lobularis* were richly crowded with broods of embryos in various stages of development.

These specimens were collected from several localities in the vicinity of Roscoff, some from the adjacent rocks of the beach, and others from Douon, a rocky island composed of Archaean schists, lying about four miles north-east of Roscoff. At one part of this island is a wonderful grotto, only accessible at low water spring tides; although open at both ends it affords sufficient shelter from the powerful currents of the surrounding sea to a thick growth of many and brilliantly coloured animals, which tapestry its walls, concealing them from sight. Besides Ascidians and Anemones, there are numerous sponges,

including *Pachymatisma Johnstoni*, *Tethya lyncurium*, a massive orange-coloured *Suberites*, various *Calcispongiæ*, and *Halisarca Dujardini* and *lobularis*.

Judging from the appearance of the grotto it would be difficult to find a more "healthy" locality for sponge growth.

The specimens when gathered were immediately placed in clear sea-water and transferred to fresh sea-water in clean "cuvettes" on reaching the station. They were not allowed to remain there for many hours, but as soon as convenient were placed in a weak solution of chromic acid, to which a few drops of osmic acid had been added (some of the specimens from the rocks of Roscoff were placed in a solution of mercuric chloride and a little acetic acid). They were finally brought into absolute alcohol, in which medium they were kept till they were cut and mounted in England. The cutting was accomplished both by the freezing and paraffin methods. In the latter case the specimens were embedded sometimes by the oil of cloves, and sometimes by the chloroform (Griesbach) process. The sections were stained by immersion first in eosin, and subsequently in hæmatoxylin; the best results were obtained when the staining was postponed till after cutting, when the sections were arranged in series and fixed to the glass slide by caoutchouc (Threlfall's method). Notwithstanding the various kinds of treatment to which they have been subjected, all the slices show essentially the same characters. The only discernible point of difference lying in the fact that slices obtained by freezing have not suffered any contraction, while those cut in paraffin have shrunk, notwithstanding all precautions, very appreciably. Since the shrinking has evidently, however, been uniform, it does not affect the characters of the embryos except as regards their apparent size. As the sections represented in the plate have all been drawn by camera lucida from paraffin-cut specimens, they are slightly smaller than they should be.

I am particular in entering into these details in order to show that the appearances which I have next to describe are not easily explicable, either as pathological or artificially pro-



duced phenomena. Some minute points of histological detail may, however, be regarded as results of treatment.

The ovum (Pl. XXXVII, fig. 1), which F. E. Schulze has traced from a wandering mesoderm cell, exhibits when 0.04 mm. in diameter a distinct external layer of structureless material, which takes a deep stain with reagents, and which appears to remain in organic connection with the surrounding matrix of connective jelly by means of short pseudopodia-like processes. Within the outer layer is a finely granular protoplasm, enclosing an excentrically placed spherical nucleus 0.01 mm. in diameter, within which again, is a spherical, transparent, highly refringent nucleolus, 0.0045 mm. in diameter. It stains deeply, and is surrounded by finely granular, scarcely-stained material, which nearly fills the sharply-contoured vesicle of the nucleus. This no doubt results from the coagulation of the liquid contents of the nucleus.

The most external layer of the cell is said by Schulze to be invisible in living specimens, and he regards it as an artificial product. No doubt its remarkably clear differentiation from the rest of the ovum is due to technical treatment; but this treatment merely brings it to light. If it existed as a separate layer in the living ovum it would be difficult to distinguish from the internal plasma, unless it differed greatly in refractive index. I am inclined to regard it as the rudiment of a blastema which we shall afterwards find existing as a most noticeable part of the segmented ovum.

The segmentation of the ovum, already known to be entire, proceeds with great irregularity. Though the stages of 2 (fig. 2) and 4 (fig. 15) are frequently in every respect normal yet just as often they present curious irregularities. In the stage 2, we sometimes find one comparatively very large, and one very small segment. A stage of three segments sometimes occurs, sometimes explicable as due to retarded division of a large segment (fig. 3), sometimes not. Stage 4 is usually normal, but one curious form is shown in fig. 4, where two opposite, not adjacent, segments remained united by a narrow bridge of protoplasm. A stage shown in section as five is not unusual, the

fifth blastomere sometimes lying in the centre of the ovum surrounded by the other four, and sometimes in a zone with them (fig. 6), when a small segmentation cavity is seen in the centre. At a very early stage a structureless blastema, at the most very finely, or scarcely at all, granular, makes its appearance. At first, as in stage 4, it merely forms a thin layer round the periphery of the ovum, becoming thicker at the junction of the segments, and sometimes extending for a variable distance between them. It then stains comparatively deeply with reagents. As development proceeds it increases in quantity and takes a less decided stain, and in the final stages of segmentation it exists as a clear, structureless matrix, which forms the outer boundary of the segmented mass, and in which the segments lie completely and separately immersed.

After the stage of five segments, seen in section, it becomes increasingly difficult to determine from sections the number of segments in the total ovum, and I will content myself with adding on this point that after seven, eight, and nine segments appear in section the number increases rapidly, till it soon becomes too great to count. A segmentation cavity is the exception, not the rule; the only instances I have met with are shown in figs. 12 and 13, and even here the cavity is partly occupied by blastomeres. In my sections a segmentation cavity does not normally occur. This is the more remarkable since Barrois, Schulze, and Metschnikoff all represent a segmentation cavity as of constant and normal occurrence. Barrois and Schulze differ, however, in details, the former finding a segmentation cavity already at stage 8, the latter not till the stage of sixteen segments has been reached.

The blastomeres are from the first remarkably granular, so that for some time they have the appearance of being a collection of yolk granules, each separately and clearly defined. The granules, at first large, gradually become smaller, rounder, and less apt to stain as the blastomeres multiply and diminish in size, till when the latter are reduced to 0.008 mm. in diameter they form the least instead of the most conspicuous part of the cell contents, while the nucleus and nucleolus, till then only

occasionally visible, now stand out as a striking deeply-stained spot (nucleolus) surrounded by a clear circular space (nucleus).

Segmentation having now resulted in the formation of a generally oval morula, consisting of a multitude of small cells embedded in a structureless blastema, a curious change follows. The several cells begin to collect together in irregular strings and heaps, forming a rude kind of network, the meshes of which are filled with the structureless blastema from which they have separated out. The process of cell aggregation continues till the cells have for the most part arranged themselves in a single external layer, forming the foundation of a blastula. At the same time a large number of the cells remain accumulated in heaps at various points on the interior of the blastula-wall. Before these have disappeared an infolding of the wall occurs at one or more points at those places, namely, where the residual cells of the interior are chiefly aggregated.

As the process of folding continues the interior of the blastula-like body soon becomes cleared of the cell-stragglers, in what precise manner, whether by their dissolution or by their deploying into line with the rest of the cells in the wall, I have no means of determining.

The singular mode of formation of the blastula and gastrula no doubt stands in close connection with the absence of a segmentation cavity in the embryo, and may be most probably accounted for as due to "abbreviation" in adaptation to development in a confined space. The blastula is not formed by the enlargement of a segmentation cavity, since this otherwise empty space can be more advantageously occupied by cells which subsequently become utilised in the formation of the gastrula. Directly a cavity becomes necessary, in order to render possible the infolding which leads to the gastrula stage, the loosely-aggregated cells of the morula pack themselves closely together to form the wall of the unfinished blastula; but as this does not afford sufficient room for them all, a part remain loosely aggregated in the interior, and these subsequently arrange themselves into a unicellular layer along the line of the infolding wall of the gastrula of which they then

come to form a part. Thus the gastrula, would appear to result from a remarkable rearrangement of the cells of the morula, the blastula stage being rapidly slurred over in order to economise space.

The commencing gastrula is seldom so normal in character as represented in fig. 19, and never retains the simple form there exhibited for long; for as the advancing hypoblast grows inwards to meet the epiblast, both become deeply folded (figs. 24 and 26), so that by the time both layers have become approximated to form a single bilamellar wall the embryo has assumed the form of a complicately folded sac (figs. 27, 28, 32).

Hence not only the blastula but the gastrula stage is slurred over, for the embryo does not wait till a gastrula form is completed before commencing to fold; but the folds arise *pari passu* with the growth of the gastrula, which thus as a definite stage in the development can scarcely be said to occur at all. The embryo which so results may be regarded as a young sponge, complete in most essential characters, save as regards the presence of pores, which are not in my sections to be observed. The outer sinuses of the folds may be regarded as rudimentary incurrent canals, and the inner sinuses as rudimentary flagellated chambers, with their ducts as yet undifferentiated, as they permanently remain in *Halisarca Dujadini*. The rest of the archenteric cavity probably gives rise to the excurrent canal system. Difficult as the history of the young sponge is, we have yet, however, by no means exhausted its complications.

Frequently, indeed generally, the young gastrula exhibits a considerable number of infoldings of approximately equal strength (fig. 18), so that at this stage, in the absence of any differentiation of the component cells (and none exists), it is impossible to determine which of the folds will give rise to the hypoblast and which will remain as epiblast. Since, however, a folded sac with a bilamellar wall is invariably the final term of the series of changes observed, one must conclude, in order to explain its formation, that one of the several commencing folds grows faster than the rest, and at length meeting them



on their inner face, unites with them to become the hypoblastic layer of the resulting bilamellar wall. In such a form as that represented by the serial sections, figs. 27 and 32, the former showing the blastopore, such a course of development can be very simply understood; but there still remain other cases in which more serious difficulties are encountered, for it appears quite possible that more than one growing fold may succeed in pushing its way into the interior, and so acquire a hypoblastic relation. The instructive series of sections shown in figs. 33 to 38 serve to illustrate such a case. Had one remained content with a single section, such as either fig. 33 or 34, one would certainly have pronounced for a definite gastrula stage, and the remaining sections would have been regarded as exceptional or abnormal. A study of the entire series proves, however, that in the case of several of the folds the adjacent walls of each have grown together and given rise to a bilaminate wall, both layers of which are of the same nature, i. e. hypoblastic. A similar union of the invaginating layer is shown in fig. 39, taken from a different embryo.

Notwithstanding all these differences in detail, and whether epiblast unite with epiblast, or hypoblast with hypoblast, the course of development invariably leads to one constant result, i. e. the formation of a folded sac, the walls of which are invariably composed of two layers of similar cells, with nothing except an almost imperceptible residuum of structureless blastema interposed between them. Probably as development proceeds beyond the stages we have observed, other cells become detached from one or both of the layers of the wall, and then wandering into the blastema, increased by growth, give rise to the mesoderm.

We have next to describe the history of the constituent cells of embryo. In the commencing gastrula (fig. 18) these are arranged in a single layer side by side to form the walls of the sac. They are longer than broad (0.004 mm. in diameter, 0.01 mm. long), with a more or less oval outline when viewed sideways (fig. 20*a*), but polygonal (by appression) when seen end on (fig. 20). A spherical nucleus (0.003 mm. in diameter)

with a spherical highly refringent nucleolus (0.0015 mm. in diameter) which stains deeply lies at the outer end or base of the cell, where also the contained granules of the cell-protoplasm appear to be most numerous. The opposite inner end of the cell terminates in a faint but clearly defined rounded margin against the structureless blastema, which fills the interior and envelopes the exterior of the embryo. Owing to the position of the nucleus the outer end of the cell is far more conspicuous than the inner, and since the cells lie evenly side by side to form the wall, it follows that this is much more darkly bordered on its outer than its inner edge.

Coincidentally with the progressive folding of the walls of the embryo the cells of both layers become modified. They multiply in number and diminish in size; at the same time the outer end becomes defined as a rounded head containing the nucleus, and constricted from the pale faintly granular body of the cell by a shallow groove (fig. 25). The cells continue to grow smaller and more numerous till the approximation of the two layers is complete (fig. 29), and perhaps subsequently. At length, soon after the formation of the double wall, the nuclei of the cells, now much reduced in size, retreat from the outer end of the cell, where hitherto they have constantly remained, towards the interior, and at the same time the outer end becomes cleared of granules, so as to resemble the collum of a choano-flagellate cell, and a flagellum is protruded. No doubt, judging from a wide analogy, a collar is also produced, but search for this in spirit specimens is ineffectual, as might be expected. The cells of both layers pass through identical changes, and are as similar at the close of the development observed as at its commencement.

On comparing the account here given of the embryology of *Halisarca lobularis* with that observed by Metschnikoff, Schulze, and Barrois, one is fairly staggered at its divergencies, not to say contradictions. As to the fidelity of the observations of these investigators no one can suggest a doubt, and as for mine the sections on which they are founded are preserved in balsam and open to inspection. Beyond the blastula

stage Schulze's observations, like those of Barrois, were made on free-swimming larvæ; indeed, in the case of Schulze's specimens development does not proceed within the parent Sponge beyond the formation of a blastula, or, at all events, of an incipient gastrula. Save for the exceptional mode of formation of the blastula our discordant results might then be explained as due to the fact that mine are obtained from intra-uterine embryos, as one might say, while Schulze's were made on free-swimming embryos. But this leads to a further inquiry, that is, for what reason should the embryos of the Roscoff Sponges remain up to later stages within the maternal tissues than those of the Sponges which Schulze obtained at Trieste? In reply to this, I would inquire whether it is not possible, indeed highly probable, that an explanation is to be found in the wide differences of condition which characterise the two localities whence the Sponges were obtained. In the Mediterranean there are no heavy tides and powerful currents, so that the Sponge larvæ can safely issue into the warm surrounding water at an early stage without fear of being washed away to sea out of reach of a holding-place, and in this early birth there is a distinct advantage to the race, for the larvæ can obtain as much oxygen and nourishment as they require from the surrounding water without putting their parent to the needless expense of maintaining them. In the English Channel, particularly about Roscoff, where the tides run high and the tidal currents are exceedingly rapid and dangerous, the case is very different; young larvæ set free too soon would be swept away from home and drifted up and down by the currents with the merest chance of finding a safe settling-place, and hence it becomes necessary that they should be prepared to attach themselves to a foreign object immediately after hatching as possible. Consequently in Sponges obtained from this locality we can trace their development up to a stage which is evidently not far remote from that of a young Sponge, and in the superfœtation thus induced by external conditions we find the key to the anomalous development we have described.

**Classificatory Position of the Sponges.**—The early appearance of flagellate, and, indeed, choano-flagellate cells in many, if not all, Sponge embryos, and their persistence throughout life as the characteristic cells of the adult Sponge, is a very remarkable phenomenon, and on the theory of heredity it meets with its most plausible explanation by supposing (as Saville Kent does) that the Sponges are directly descended from the choano-flagellate Infusoria. It is difficult to suppose that such complicated structures as the choano-flagellate cells of the sponge should so closely resemble the choano-flagellate Infusoria and yet be of independent origin.

On the other hand, the Sponges are quite clearly not Protozoa; the individuality of the component cells of the Sponge is subordinated to that of the total organism to an extent and in a manner which meet with no parallel amongst the Protozoa; in the lowest Sponges a definite epithelial layer, together with a mesoblastic connective jelly, always coexists with the flagellate cells, while in the higher Sponges certain cells are differentiated into muscle-fibre-cells which are remarkably similar to the involuntary muscle-cells of the higher animals, and which are arranged in definite muscular sphincters; at the same time other cells are differently modified to form a tissue which curiously simulates fibrous connective tissue. Indeed, so far as tissue differentiation is concerned, a geodine Sponge is, on the whole, a more highly organised animal than a fresh water Hydra. In the reproduction of the sponge, even in such forms as *Halisarca*, by ova and spermatozoa we meet with another metazoan character.

It makes no difference if some Sponges develop parthenogenetically,<sup>1</sup> of which at present we have no proof, since parthenogenesis frequently occurs among the higher Metazoa. No argument is to be drawn from the segmentation of the ovum, since very regular segmentation occurs in the product of conjugation of some Protozoa; but the arrangement of the resulting cells in two layers, and the formation of a gastrula,

<sup>1</sup> I expect that parthenogenesis will be found to be of much more frequent occurrence among the lower Metazoa than is at present suspected.

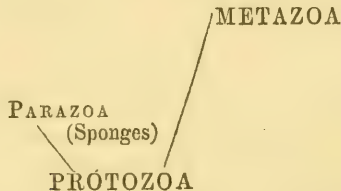


either by invagination or delamination, is a remarkable point of resemblance between the Sponges and the Metazoa.

Indeed, on summing up the resemblances between Sponges and Metazoa one inclines to remain in doubt whether it is more probable that the metazoan or the infusorian characters should have been independently evolved. On the whole, I incline myself to regard the metazoan characters as "homoplastic," to use Lankester's term, and the infusorian as phylogenetic. The two chief metazoan characters are the presence of both sexual elements and the formation of a gastrula. With regard to the former we know next to nothing as to the causes which have led to sexual differentiation, but we have every reason to suppose that it has originated independently in animals and plants, and if so it might just as well have done so in the case of Sponges and Cœlenterates. The formation of the gastrula is a different matter, and here it is to be remarked, first, that the choano-flagellate cells of the Sponge make their appearance very early in development, before the formation of a gastrula, as is clearly the case in the amphi-blastula of *Sycandra raphanus*; secondly, folding is one of the commonest phenomena in various stages in the development of all animals, and is probably susceptible of a very simple mechanical explanation; at all events in a large number of cases similar foldings originate quite independently of heredity, and give rise to homoplastic organs which are certainly not homologous. It is scarcely necessary to cite cases, they are so numerous; but one may instance the formation by folding of the eye in Molluscs, Worms, Peripatus, and Vertebrates as a case in point. Furthermore, both in Sponges and Cœlenterates the formation of the gastrula takes place in two ways, by invagination, as in *Sycandra*, and by fission of the mesenchyme, as in *Plakina* among Sponges and *Eucopa* among Cœlenterates. Its origin in at least one of these ways is inexplicable directly from heredity. For if for the sake of argument we assume both Sponges and Cœlenterata to have had a common ancestor, a gastrula which originated by invagination, we must then suppose that the formation of a gastrula by fission in each group arose as an

independent modification, due, no doubt, to the similar action of physical causes. If the original gastrula was formed by fission then those secondarily produced by invagination are independent modifications. But if secondary gastrula, having considerable similarity to each other, have thus been produced independently from a primitive gastrula, it becomes quite possible that the primitive gastrulæ themselves are independent modifications of groups of cells originally possessing very different characters. It is also not to be forgotten that the divergencies between the development of Sponges and Cœlenterates are almost as striking as the resemblances. It may be objected to this line of argument that in the Sponges originally similar cells have become differentiated into muscle fibres and connective-tissue fibres which are homoplastic but not homologous with those of the true Metazoa, and hence that independent origin of similar cells is just as probable as the independent origin of similar organs. Admitting this, one would point out that the choano-flagellate cells of the Sponge appear early in its embryological history and persist as its special characteristic, while the muscle and connective-fibre cells appear comparatively late in life, and are produced by the Sponge to meet special exigencies.

It will appear, therefore, according to the view here advocated, that while the Sponges are evidently homoplastic with the Metazoa they are of independent origin, having arisen as a separate phylum from the choano-flagellate Infusoria. For this phylum I propose the name Parazoa; the relations of the three groups into which the animal kingdom will then be divided are expressed in the diagram below.



The gastrula of the Parazoa is essentially distinguished from

that of the Metazoa by the fact that in it the hypoblast consists of collared flagellate cells.

Perhaps I may be allowed to add some remarks on a possible mode of origin of the Spongiæ.

It is generally admitted that one must begin one's suppositions with a colony of Protozoa. Let us then assume the existence at some early period of a closely aggregated colony of choano-flagellate Infusoria, which need not at first have been free-swimming. Encystment in some form or other is frequent in this group of Infusoria, and one may suppose that at some point in its life cycle the members of our choano-flagellate colony passed through a resting stage in which they existed as amœboid cells, enveloped in a gelatinous cytoblast.

If now some of the flagellate individuals became "enjelled" —one can hardly say encysted—at a different time to the rest, the colony would become differentiated into a set of cytoblastic and a set of flagellate individuals. This unstable differentiation might very probably prove of advantage to the colony, saving it from becoming wholly inactive at any given time, for while some individuals rested others would remain on duty securing food for the cœnobium.

Some such stage as this appears in point of fact to exist, if we may trust Mr. Saville Kent's description (which certainly stands in need of confirmation) of that compound flagellate Infusorian which he has named *Protospongia Hæckeli*.

We may next suppose that the individuals of the colony which remained active derived some special advantage from the existence of the cytoblastic portion of the colony; possibly it led to a slight economisation of energy by retarding perhaps loss of heat, perhaps osmotic action. By this economising of energy the active individuals may have been relieved from the necessity of encystment, and, growing more rapidly than the cytoblastic portion of the colony, may have formed a continuous layer over it. A double advantage would follow from this, for the increase of mass of the colony not involving increase of surface would necessarily lead to some slight saving of heat, and the cytoblastic portion would both serve as a layer

of support and as a store of nourishment in reserve. The differentiation would now become permanent and stable, both the cytoblastic and the flagellate cells finding themselves advantaged. In this state the compound infusorian might have existed for a considerable interval, reproducing itself by the subdivision of the cytoblastic cells into flagellate offspring. The work of reproduction in course of time devolving upon some of the better-fed cytoblastic cells, instead of being performed by all: the Infusoria, resulting from the metamorphosis of each ovum, might in time become more numerous, and remaining aggregated produce a free-swimming globular cluster of Infusoria.

It is in every way probable that several such agamic ova would be present in a single colony at the same time, and that they would coexist in various stages of maturity. Eventually one may suppose that the young flagellate cells arising from the division of one ovum bored their way into the vicinity of another ovum, mature, but not yet in process of division, and penetrating its substance, conferred upon it that advantage which results from fertilisation. It is interesting in this connection to find that the young infusorian produced by a choano-flagellate parent is at first without a collar and contractile vesicle, and of much smaller size than the adult. So similar is it apparently to a Spermatozoon that S. Kent was led by this similarity to throw quite unjustifiable doubts on the real existence of spermatozoa in the Sponges.

The ovum fertilised by the young flagellate cell (ancestral form of Spermatozoon) might very naturally, owing to the increased energy conferred upon it, have proceeded farther with its metamorphosis while still within the parental tissues than had previously been the case. If at the same time it continued to receive nourishment from the parent stock it might be some time before segmentation was carried far enough to produce cells small enough for metamorphosis into flagellate collared cells, and in order to secure to each cell of the dividing mass its fair share of nourishment it is possible that each may have worked its way to the exterior, so as to



produce a segmentation cavity and a blastula form. This may not have been found necessary in all cases, and the solid morula, like that of our *Halisarca* embryos, may represent such an exceptional dispensation with the early blastula form.

From this point the course of development probably diverged in different groups of Sponges. In some a hollow blastula of flagellate individuals was produced, and this either owing to osmotic action or unequal growth at different parts of its periphery became infolded at one point or more. The infolding once commenced would proceed, *pari passu*, with the growth and subdivision of the invaginated cells. If dependence is to be placed on our *Halisarca* embryos one may suppose that several foldings were in some cases produced. The sinuses of the folds would probably serve as quiet recesses in which food particles would be collected. Anyone watching an infusorian at work must have been surprised at the few particles of food it secures compared to the currents it sets in motion. There is evidently here a great waste of energy, and possibly some of this is spared by the formation of a gastrula. But the currents produced by flagella in a sac open only at one end would become less effective as the sac became more complete, and would cease to be of any use in obtaining food as soon as the gastrula attached itself to some foreign body by its oral end. Hence the development of pores which restore communication with the exterior, though it is difficult to understand how the pores arose.<sup>1</sup> Subsequently the mesoblast would arise by migration of the endodermic cells.

In yet other groups of Sponges, probably the most numerous, the blastula buds off internally cells which give rise to mesenchyme, comparable to the cytoblast of the ancestral infusorian colony, and thus the ancestral form of the Sponges appears at an early stage. The gastrula cavity is now formed by an internal splitting of the mesenchyme, and this need not neces-

<sup>1</sup> Mérijowski describes pores as perforating the wall of the blastula of the embryo of *Obelia* ('Bull. d. l. Soc. Zoo. d. France,' 1883, p. 27, Extrait). As they, however, do not occur in the gastrula, they cannot be homologous with the pores of Sponges.

sarily be a modification of the process of invagination, but a wholly different method of arriving at the same end and resulting from the presence of a mesenchyme. For a hollow blastula is evidently in a very unstable state, and folding might almost be predicted for it; but in a planula containing mesenchyme the internal layer is well supported, and not so advantageously conditioned for folding.

If, however, in the planula the flagellate layer grows more rapidly than the mesenchyme can keep pace with it, and yet at the same time maintains its coherence, and remains adherent to the mesenchyme, tension will arise within the latter, from which on account of its extreme tenderness it would readily relieve itself by splitting.

The fissure once formed, whether for this reason or any other, soon becomes lined by a layer of flagellate collared cells, the appearance of which is remarkable, but readily intelligible, if we adopt the hypothesis that the cells of the mesenchyme have a flagello-choanate infusorian ancestry.

In conclusion I would offer some comment on the classificatory relations of *Halisarca*. As a genus it is distinguished by the comparatively undifferentiated character of its cells; the ectodermal cells retain their flagella—though not the collar—throughout life, and the mesodermal cells exhibit none of those modifications which are so characteristic of the higher Sponges, such as the *Geodiæ*. There is also a conspicuous absence of a skeleton, siliceous or calcareous. This striking simplicity of both species of *Halisarca*, and the absence of any features in their development which would tend to show that they are degraded forms, leads me to regard them as amongst the lowest of existing Sponges. On the other hand the *Chondrosiadæ* which have been associated with them appear to occupy a much higher position. They suggest affinities with the *Tetractinellidæ*, of which, perhaps, they may be regarded as degraded descendants.

Consequently, however much simplicity of classification may suffer, it appears to me advisable to remove the *Chondrosiadæ* from the order *Myxospongiæ*, which will then contain

only the genus *Halisarca*, and this should be divided into two, for *Halisarca lobularis* and *Halisarca Dujardinii* differ far more from one another than is consistent with specific distinction merely. A fundamental difference is to be found in the character of the canal system in the two Sponges, and to this may be added the difference in the character of the ectoderm and the presence of skeletal fibres in *H. Dujardinii*, which are absent in *H. lobularis*. But it is needless to insist on this point further, as I find since writing this paper that Vosmaer<sup>1</sup> has already conferred a new generic name (*Oscarella*) on *H. lobularis*.

March, 1884.

## EXPLANATION OF PLATE XXXVII,

Illustrating Mr. W. J. Sollas's Paper "On the Development of *Halisarca lobularis*" (O. Schmidt).

The figures were all drawn with camera lucida, but since the paper on which they were traced was placed considerably below the level of the microscope stage they are enlarged to nearly twice the diameter that the lenses used for each would otherwise indicate.

FIG. 1.—Mature ovum. Zeiss, D Obj., No. 4 oc.

Figs. 2 to 14 and 16 represent sections illustrating various stages in the segmentation of the ovum. All drawn with a Ross 1-inch Obj., Zeiss, No. 4 oc., except Figs. 8, 9*a*, 14*a*, and 16*a*.

FIG. 2.—Stage of two segments, with structureless blastema shown on each side of the cleavage plane.

FIG. 3.—An exceptional stage of three segments.

FIG. 4.—An exceptional stage of four segments, showing two opposite blastomeres united.

FIG. 5.—Normal stage of four segments, with surrounding blastema.

FIG. 6.—Section showing five segments.

FIG. 7.—Section showing six segments, with surrounding blastema.

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<sup>1</sup> Bronn's 'Thierrisch,' Bd. ii, pl. viii, article "Porifera."

FIG. 8.—A single blastomere of Fig. 7. Zeiss, E Obj., No. 4 oc.

FIG. 9.—Section showing nine segments. 9a, a single blastomere of Fig. 9. Zeiss, D Obj., 4 oc.

FIG. 10.—A section showing nine segments.

FIG. 11.—Embryo showing traces of a segmentation cavity.

FIG. 12.—Embryo more advanced, with traces of a segmentation cavity.

FIG. 13 and 14.—Embryo with numerous blastomeres, but no segmentation cavity.

FIG. 14a.—A single blastomere. Zeiss, E Obj., No. 4 oc. Diameter of this blastomere 0.02 mm., of the nucleus 0.005 mm., of the nucleolus 0.002 mm.

FIG. 15.—Latest stage of the solid morula; the pear-shaped outline is exceptional.

FIG. 15a.—A single blastomere of Fig. 15. Zeiss, E Obj., No. 4 oc. Diameter of blastomere 0.01 mm., of nucleus 0.0025 mm., of nucleolus 0.0008 mm.

FIG. 16.—Embryo in which the blastomeres of the morula are beginning to arrange themselves along definite lines. Zeiss, D Obj., 3 oc.; reduced one half.

FIG. 16a.—Several cells of the same embryo. Zeiss, E Obj., No. 4 oc. Diameter of cells 0.006 to 0.01 mm., of nucleus 0.0018 mm., of nucleolus 0.0006 mm.

FIG. 17.—Embryo a stage further advanced, showing the alignment of the cells to form a definite wall surrounding an irregular internal cavity. Zeiss, E Obj., No. 3 oc.; reduced one half.

FIG. 17a.—Constituent cells of Fig. 17. Dimensions: length, 0.012 mm.; breadth, 0.0045 mm. Zeiss, E Obj., No. 4 oc.

FIG. 18.—Embryo with the external wall definitely constituted. Ross 1-inch Obj., No. 4 oc.

Figs. 19 to 39, showing the further progress of the embryo, are drawn with Ross's 1-inch Obj. and Zeiss's No. 4 oc., except Figs. 20 and 20a, 24 and 24a, 29, 30, and 31.

FIG. 19.—Section of gastrula, the most normal form observed.

FIG. 20.—Constituent cells seen *en face*. Fig. 20a, seen laterally. Zeiss, H Immersion Obj., No. 3 oc. Dimensions: length, 0.01 mm.; breadth, 0.004 mm.; nucleus, 0.003 mm. in diameter.

Figs. 21 to 23.—Three sections of one and the same gastrula.

FIG. 24.—Section showing the folding of the wall of a gastrula; the outer sinuses of the folds represent commencing incurrent canals; the inner sinuses, derived from the archenteron, the commencing excurrent system.

FIGS. 25 and 25a.—Constituent cells, seen sideways and *en face*. Zeiss, E, Obj., No. 4 oc. Dimensions of cells: length, 0.008 mm.; breadth, 0.003 mm.

FIG. 26.—Gastrula still further folded.



FIGS. 27 and 28.—Sections of the same embryo in the last stage of folding observed.

FIG. 29.—Constituent cells of the hypoblastic and epiblastic layers of the wall of the embryo, shown in Fig. 28. Zeiss, E Obj., No. 4 oc.

FIG. 30.—Cells of a similar embryo, more advanced. The nucleus has retreated to the inner end of the cells, and a flagellum has been produced.

FIG. 31.—One of the foregoing cells, more highly magnified.

FIG. 32.—A third section of the same embryo as that from which Figs. 27 and 28 were obtained.

FIGS. 33 to 38.—A series of sections of a single embryo, disclosing irregularities which would not have been conjectured had the section Fig. 33 alone been observed.

FIG. 39.—Section of another abnormal gastrula.

## A Contribution to the Knowledge of Rhabdopleura.

By

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With Plates XXXVII *bis*, XXXVIII, XXXIX, XL, XLI.

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RHABDOPLEURA was discovered by the Rev. Alfred Merle Norman, in deep water (90 fathoms) off the Shetlands, in 1868. The original specimens were described by Professor Allman in this Journal, vol. ix, 1869, p. 57, and many of the remarkable characters of the new genus which he founded for the reception of this form were fully recognised and discussed by him. Subsequently Prof. G. O. Sars published (Christiania, 1872) an account of Rhabdopleura, based upon specimens obtained at a depth of 200 fathoms off the Lofoten Islands, and studied by him in the living state. Sars's memoir was reproduced in this Journal in 1874 (vol. xiv, p. 23). The specimens studied by Sars were regarded by him as belonging to a different species from that described by Allman, for which he proposed the name Rhabdopleura mirabilis, Allman having named his species Rh. Normani after the eminent naturalist, who detected it in the contents of his dredge, and sent it to Allman for study. I spent part of the summer (end of July to beginning of September) of 1882 at Lervik, on the Island of Stordoe, at the mouth of the Hardanger Fjord, near Bergen in Norway, in order, among other things, to obtain living specimens of Rhabdopleura, in the hope of clearing up certain doubtful

points in its anatomy, and, if fortune favoured me, of obtaining material for the study of its development from the egg. In 1879, Dr. Norman had for a second time encountered *Rhabdopleura*, having dredged it at this spot (Lervik) in water of 150 fathoms' depth, growing upon the dead parts of the corallum of the beautiful *Lophohelia prolifera*, which is so abundant on this coast. Dr. Norman very kindly gave me minute instructions as to the exact spot at which he had made this second find of *Rhabdopleura*, and within a few days of establishing myself at Lervik I was successful in obtaining some specimens.<sup>1</sup> A little later, Dr. Norman himself joined me at Lervik and gave me the immense advantage of his help in dredging operations. We very soon obtained *Rhabdopleura* from a depth of 40 fathoms only, and growing not upon a hard corallum, but upon a much more favorable substance for the purposes of study, namely, on the test of *Ascidia mentula*. At the same spot, at the very mouth of the harbour of Lervik, south of the lighthouse island, we obtained a specimen (the fourth on record) of the Bonellia-like Gephyrean *Hamingia arctica*, which was remarkable not only for its occurrence south of the Arctic Circle and in comparatively shallow water, but for retaining its præoral appendage in full perfection (see 'Ann. and Mag. Nat. Hist.,' Jan., 1883).

From this spot I was able then, on many succeeding days, to obtain a supply of *Rhabdopleura*. Unfortunately, the period of sexual reproduction appeared to have passed (it was then August), and not only were there no ova or embryos to be found, but the adult polypides seemed to be, in the majority of examples, in a moribund condition. Nevertheless, I was able

<sup>1</sup> I must not omit to express my warm thanks to Mr. Herman Friele, of Bergen, who spared no trouble in helping me to make my arrangements at Lervik, and gave me the benefit of his long experience in exploring the Bergen district. To him, as well as to the veteran naturalist Danielssen, and to Armauer Hansen, I am indebted for the most agreeable memories of the hospitable Norse city—a city truly remarkable to the naturalist for its splendid zoological museum and the circle of eminent men who give their time to its enrichment.

to find some which were active and healthy, and to make some observations of value with reference to (1) the existence of an internal cartilaginous skeleton, (2) the existence of a body cavity (denied by Sars), (3) the position and characters of the testis and spermatozoa, (4) the nature of the axial chitinous rod or blastophor of Allman, and (5) the general features of the bud-production and constitution of the colony. I also prepared some of the specimens by treatment with corrosive sublimate, followed by alcohol; and of these, sections were subsequently cut in my laboratory in London by my assistant, Mr. A. G. Bourne, who accompanied me in my visit to Lervik and rendered me much valuable aid.

Assuming an acquaintance on the part of the reader with the memoirs of Allman and Sars, I shall describe seriatim the matters of interest which my observations have yielded.

#### THE TUBARIUM.

In Plate XXXVII *bis* are drawn two specimens of colonies of the *Rhabdopleura* obtained at Lervik. They are represented as magnified about three times linear. Both specimens are attached to the test of *Ascidia*. The polypides are withdrawn within the tubes which they secrete and inhabit; consequently, we have practically before us merely the branched tubular body forming the dwelling or house of a *Rhabdopleura* colony. It seems undesirable to apply the term "cœnœcium" to this structure, or the corresponding term "zoœcium" to the units of which it is composed, since those terms are applied in the description of Polyzoa to structures which, though acting in a sense as dwellings for the polypides, have a totally different origin and morphological significance. The zoœcium of an ordinary Polyzoon is the locally thickened cuticle of the hinder part (antitentacular region) of the polypides body to which it is permanently adherent. The tubes of *Rhabdopleura* have no such origin or attachment. Each tube is built up of a series of rings, and each ring is separately secreted and added



to its predecessors by the so-called buccal shield or præoral lobe of the polypide. The structure of the tubes is shown in Plate XXXIX, figs. 1 and 4. On examining the tubarium of *Rhabdopleura* in order to discover the law of its growth, we find that it consists of a branching axis (Pl. XXXVII *bis*, figs. 1 and 2, *b*), carrying throughout its length numerous lateral appendages standing out from it, sometimes on one side, sometimes on the other. These lateral appendages have the form of long tubes with circular non-dilated mouths. The axis presents no characters by which it can be distinguished into primary, secondary, and tertiary portions, the branches having always the same character as the stock by the forking of which they arise. The axis is closely adherent to the Ascidian test throughout its length, and is often encrusted in its older parts by foreign growths. The polyp-tubes form conspicuous objects from the fact that they are attached like the axis itself for half their length to the ascidian-test, and then, bending at a right angle, stand up perpendicular to the surface of attachment. In the figure these perpendicular tubes are represented without shading. No numerical law as to the position of branches or the number of polypide tubes given off between one bifurcation of the axis and another can be formulated. Branches usually arise by bifurcation, but three or even four branches may sometimes originate in very close proximity to one another. The arborescent form is accordingly indefinite. The free extremities of the branches present two different conditions. A branch often terminates in an upstanding polyp-tube (see Pl. XXXVII *bis*, *d*). Such a branch is a "completed branch," and is not in a condition to produce any further polypides and polyp-tubes. On the other hand, in many branches we can trace the axis to a considerable distance beyond the last upstanding polyp-tube (Pl. XXXVII *bis*, *a*), pursuing a very straight course and closely attached to the Ascidian test. This is a "growing branch." When examined as to its contents, it is found that buds destined to grow into numerous polypides are being formed within this straight recumbent termination of the branch, and that it is

itself being daily extended by the addition of segments to its free extremity (see fig. 1, *a*, Pl. XXXIX).

The *Rhabdopleura mirabilis* of Sars does not quite agree with the above description, since the polypide tubes are for no part of their course recumbent, but spring directly from the axis at right angles to it. It is exceedingly probable, however, that this difference is one due to the nature of the surface upon which the *Rhabdopleura* is growing. The *Rh. mirabilis* of Sars was taken by him in fragments growing upon coarse sand—attached not to a continuous support, but to one piece after another of coarse angular rock-particles. The Lervik specimens agree in the character of their polyp-tubes with Dr. Norman's original Shetland specimens, with which I have been enabled by his kindness to compare them. The latter were attached to dead shells, and one of the finest specimens which I dredged at Lervik was spread over the dead shell of a large *Pecten*. There is no doubt then, that the Lervik species is the *Rh. Normani* of Allman.

I do not think that Sars has given sufficient reason to lead to the conclusion that his *Rh. mirabilis* is anything more than a variety of *Rh. Normani*, determined by the character of its support.

The tubarium of *Rhabdopleura* is formed from a transparent substance of horny consistency. When a colony is examined with the microscope it is found that the whole tubarium is built up of separate segments. In the free up-standing polypide tubes these segments have the form of complete rings, segments of a cylinder cut at right angles to its long axis, which, to use a homely comparison, resemble a number of table-napkin-rings piled one on the top of the other. Under favorable circumstances these rings can be detached from one another. Each ring is the work of one period of activity on the part of the buccal-shield of the polypide inhabiting the tube, and the older the polypide the more numerous will be the rings of the tube added from time to time to the mouth of the tube (Pl. XXXIX, fig. 1, *y*).

On the other hand, the segments which constitute the

attached or recumbent portions of the tubarium are not true rings, but are formed by two obliquely-set pieces, which appear to overlap one another. Gradations between the extreme form of two oblique half-segments and a single complete seamless ring are found (Pl. XXXIX, fig. 4). This difference in the structure of the proximal and distal portions of the tubarium is simply due to the form of the buccal-disc by which they are secreted. The complete rings of the upstanding tubes are secreted by the complete circular buccal-disc of a fully-grown polypide (Pl. XXXVIII, figs. 1 and 5). The curious obliquely-divided segments of the recumbent portion of the tubarium are always secreted by an immature polypide, such as that seen at *a*, fig. 1, Pl. XXXIX. In the immature polypide the buccal-disc has, as pointed out by Allman, a very curious bilobed form. The axial portions of the tubarium are necessarily always formed by what may be termed the "leading" bud of each branch, and, similarly, the proximal portion of each polyp-tube is formed by the polypide when in a young condition. The assumption of the adult form, either by the leading bud of a branch, or by any one of the numerous lateral buds of a branch, coincides with the change of direction of growth of the corresponding part of the tubarium, viz. it proceeds to grow at right angles to the surface of attachment; it coincides also with the production of complete ring-segments, in the place of the obliquely-notched pieces.

A further examination of the tubarium of *Rhabdopleura* shows that it is divided internally by transverse septa (Pl. XXXIX, fig. 1, *p*) into a number of separate chambers. These septa occur in the various branches of the axial portion of the tubarium, and are so placed that a portion of the axial tube giving off a single polyp-tube from its side is shut off from similar portions in front and behind, each of which also gives origin to one polyp-tube.

The significance of this arrangement is seen when the terminal portion of a budding branch is examined (fig. 1, *a*, and fig. 7, Pl. XXXIX). It is then seen that the buds lie one behind the other in this portion of the axis, and that they are

placed each in a completely closed chamber formed by the growth of septa across the tube. When a bud reaches a certain stage in development it breaks through the wall of its chamber and grows outwards in a direction forming a sharp angle to the axis. At the same time the young bud which thus bursts its prison wall forms a ring around the orifice of rupture, and upon this a second, third, fourth, and so on, building up its polyp-tube as it advances in growth (see Pl. XXXIX, fig. 1, *ee*). Thus each polyp-tube is connected with a distinct chamber of the axis. It occasionally happens that the young bud enclosed in an axial chamber of the tubarium does not break its way out, but atrophies. Thus are produced the "sterile chambers" noticed both by Allman and Sars. In the specimens studied by me such chambers occurred (Pl. XLI, figs. 2, 6), but were very rare. It is probable that they are produced more abundantly at one period of the growth of the colony than at another.

Both the axial portions of the tubarium and the polypide-tubes may exhibit very sudden turns and twists. One of the most remarkable cases of coiling of the tube which I have seen is drawn in Pl. XXXIX, fig. 2.

The living colony, which makes for itself the tubular habitation just described, should perhaps have been dealt with before the latter. There are advantages in both methods of treatment, for it is easier to understand one when the other has been already examined, whether we commence with tubarium or zoarium.

### THE POLYPIDES.

It will be convenient to note first of all various points in the structure of the individual polypide, and then to revert to the subject of bud-production and the method of connection of the aggregate of polypides known as a colony or zoarium.

General Form.—A surface view, with natural colours, of a polypide of *Rhabdopleura Normani* is given in Plate



XXXVIII, fig. 1. We distinguish first of all the polypide-stalk or "gymnocaulus" from the polypide body. The "gymnocaulus" was called "contractile cord" by Sars, and was erroneously identified by Allman with the "funiculus" or posterior mesentery of the Phylactolæmous Polyzoa. Allman was led to use this term and to make this identification through the imperfectly preserved condition of his specimens, which led him to believe that a body wall existed outside what is the true body wall of Rhabdopleura, adherent to the tubarium as is the body wall of a common Polyzoon to its cuticular product the zoëcium. The body wall of Rhabdopleura has not these relations, and the polypide-stalk has no relation to the funiculus of other Polyzoa. The "body" of the polypide (as opposed to the gymnocaulus) presents us anteriorly with the buccal-shield or disc, a præoral muscular expansion between mouth and anus. It occupies the same position as the epistome of Phylactolæma and Phoronis. It attains even more remarkable proportions in the allied genus Cephalodiscus of McIntosh than in Rhabdopleura: it is active as a locomotive organ, serving apparently to raise the polypide in its tube so that it may partially emerge from the tube's orifice; and also it is active as a secreting organ, building up ring after ring at the mouth of the tube. The forms assumed by the buccal-shield in various conditions of expansion and contraction, as well as the distribution of pigment upon its surface, may be gathered from Plate XXXVIII and its explanation. It is covered with fine cilia, which occur also on the lophophoral filaments, the rest of the surface of the animal being devoid of cilia. Beneath the buccal-shield, on that face of the body to which the stalk is attached, is the mouth. A pigmented region of the body, immediately adjacent to the mouth, may well be called the thorax (Pl. XXXVIII, fig. 1, *F.*). It is followed by a sac-like region, the epidermic cells of which are mostly devoid of pigment, and consequently allow the yellow colour of the intestine to show through. This region may well enough be termed the abdomen (*I.*). The characteristic Polyzoon-bend of the intestine can be traced in this region of the body, and the anus (*B*) is placed on a papilla which projects

from its ab-oral face, near the limit between it and the thorax. It is to the oral face of the abdomen that the gymnocaulus is attached, or rather it is at this spot that the polypide narrows to the form of a stalk. Diverging right and left of the body from the thorax are the two branchial arms, or lophophor-arms as they are best termed in view of the fact that they are the same organs as the arms of the hippocrepian lophophor of other Polyzoa (Pl. XXXVIII, figs. 1, 2, *Ga.*). These carry each two rows of ciliated filaments, usually fifteen in a row, though I have sometimes observed a few more or less. On the aboral surface of each lophophor arm, at its junction with the thorax, Sars discovered a ciliated tubercle (Pl. XXXVIII, figs. 2, 3, *K.*), which is possibly related to the osphradium of Mollusca, since that sense-organ occupies a similar position in relation to the ctenidium; and it is not improbable, whatever view may be taken of the relations of other regions of the body of Rhabdopleura to the regions and body-lobes of Mollusca, that the ctenidia of the latter are the genetic equivalents of (homogenetic or homologous with) the lophophoral arms of Polyzoa.

**Pigment.**—The epidermic cells which clothe these various regions of the body of Rhabdopleura develop at intervals black and orange-brown pigment, so as to give a spotted leopard-like appearance to the animal, which is represented for the first time in the coloured drawings accompanying this memoir. The epidermic cells are either colourless, entirely brown, or entirely black (see Pl. XLI, fig. 1). They have a very definite arrangement, and the coloured cells are especially abundant on the thorax, buccal-disc, and lophophoral arms and tentacles. The coloured cells are deficient on the abdomen, but are more or less abundant on the contractile gymnocaulus.

An accumulation of fine spherical pigment corpuscles at the superior dorsal margin of the buccal-disc must be regarded as a rudimentary special sense organ for the perception of light (see Pl. XXXVIII).

**Alimentary Canal and Cœlom.**—The sac-like abdomen is very closely fitted by the wide stomach and reflected intes-

tine described by both Allman and Sars. The yellow cells which line the stomach are ciliated (Pl. XLI, fig. 8). I have no special remarks to make with regard to the form or divisions of the alimentary canal. It is seen in the drawings of the sections given in Pl. XLI, figs. 9—13. But in those sections it will be observed that a space, though not a large one, intervenes between the body wall and the wall of the alimentary canal (*m*). This is the body cavity or *cœlom*, the existence of which has been expressly denied by Prof. G. O. Sars. It is easy to see the body cavity without having recourse to the method of sections. Living specimens under slight compression show it clearly enough on the oral face of the abdomen in the neighbourhood of the attachment of the stalk. It can also be distinctly traced into the gymnocaulus or polypide stalk (Pl. XL, fig. 12, B. c.).

The tissue which bounds the body cavity consists of fusiform cells tapering into fine fibres, sometimes branched. These can be detected both on the wall of the stomach and on the inner face of the body wall, and occasionally they are seen stretching from the stomach wall to the body wall. I have not been able to detect any free floating corpuscles in the body cavity, nor have I definitely traced it as a wide space into the lophophoral arms and buccal-disc; but it can hardly be doubted that in the form of fine spaces between the deep tissues there is such an extension of it.

**Skeleton.**—One of the first facts of interest which I was able to add to our knowledge of Rhabdopleura, as represented by the memoirs of Allman and Sars, was the existence of a consistent and extensively developed internal (mesoblastic) skeleton. This consists of two chief parts, the skeleton of the lophophoral arms (Pl. XL, fig. 1), and the skeleton of the contractile cord (Pl. XL, figs. 5, 11), or polypide-stalk, or gymnocaulus. The substance of which the skeleton is composed appears to be of a cartilaginous consistence, and resists decomposition more readily than the epidermic tissues, and can, consequently, be readily demonstrated in a specimen which is commencing to break up after death.

The skeleton of each lophophoral arm consists of a portion corresponding to the arm itself, which expands in the region of the thorax into a thin plate, and attached to this—apparently articulated to it—the skeletal axes of the two rows of filaments. I was not able to detect any definite cell structure in the skeletal tissue, but it has a refringency indicating a certain density, and presents small twisted filaments and particles within its substance at intervals. It resists the action of weak acids and alkalies.

The existence of this skeleton is no doubt connected with the very varied and free movement which each tentacle exhibits, but the muscular cells related to it and to them cannot be specially identified. In Professor McIntosh's *Cephalodiscus dodecalophus* (dredged by the "Challenger") I have satisfied myself that a precisely similar skeleton exists. The tentaculiferous arms of that form are extremely similar in all respects to those of *Rhabdopleura*, excepting that they are numerous instead of being limited to a single pair, and have the free end terminating in a curious knob.

The skeleton of the contractile polypide-stalk consists of an axial cord (of the same tissue as that forming the lophophoral skeleton), which extends from the sides of the abdomen, where the stalk originates, to the termination of the soft portion of the stalk, at the point where that organ is cuticularised, and converted into Allman's blastophore. It is continued indefinitely along the cuticularised stalk in a modified condition, being shrunken and histologically metamorphosed. In connection with this skeletal cord it is easy to demonstrate the presence of a muscular band consisting of closely-set fusiform cells (Pl. XL, fig. 5, *c*). This band lies on one side of the skeletal cord, viz. on that furthest from the polypide's body. Consequently, when it contracts it tends to throw the soft, thin-walled stalk into coils. The extension of the polypide from its tube necessitates a complete uncoiling and stretching of the stalk, and is effected partly through the elasticity of the skeletal cord straightening itself as the muscles relax, and partly, as G. O. Sars has pointed out, by the active progressive



movement of the polypide due to the cilia, and possibly to the very slight undulating movements of the buccal-shield.

**Testis.**—No reproductive organs were found, either by Sars or by Allman, in *Rhabdopleura*. McIntosh found very large ova in *Cephalodiscus*, but no testis. I have found a well-developed testis in *Rhabdopleura*. Its position, shown in Pl. XL, fig. 7, is remarkable, since it projects from the surface of the body, stretching the delicate integument of the abdomen. It lies parallel to the intestine on the right side, opens near the anus, and in some cases reaches back beyond the rounded end of the abdomen, projecting as a lobe beyond. It was present in only a few of the polypides examined by me at Lervik in August; and I infer that they were exceptional specimens, in which the testis had ripened late, or for other reasons had not discharged itself during the breeding season, which probably was just over when I began my studies. Accordingly, I should urge anyone who may wish to attempt to obtain a knowledge of the embryology of *Rhabdopleura* to commence dredging operations in the middle of June.<sup>1</sup>

The testis of *Rhabdopleura* has the form of a much elongated sac, ending blindly at one end and opening by the other to the exterior by a special pore. At the blind end some sperm mother-cells were seen, but the greater part of the sac, in all cases, was densely packed with ripe spermatozoa, which could be discharged by pressure at the genital pore, and then exhibited active movements (Pl. XL, fig. 8).

This is a remarkable form of testis, and unlike anything known in the ordinary Polyzoa. The sac is possibly to be regarded as a hernia-like protrusion of the body wall. The position of the orifice corresponds with that of the genital ducts of *Phoronis*, but these are modified nephridia. On the contrary,

<sup>1</sup> I may mention that last year, I employed Rasmus Lilljebo of Lervik, who had been one of my boatmen in the previous year, to dredge for *Rhabdopleura* in May, June, and July, in order, if possible, to ascertain its breeding season, in case I should be able to go again myself in some future year to Lervik. He did not succeed in obtaining a single specimen of *Rhabdopleura*, either with or without sexual organs.

there is no suggestion of a nephridium about the testicular sac of Rhabdopleura. It belongs to that class of gonads (ovaries and testes) which I have elsewhere ('*Encycl. Britann.*,' art. "*Mollusca*," p. 682) distinguished as idiodinic (contrasted with the nephrodinic). The Mollusca are in this case, whereas the Polyzoa generally, the Brachiopoda, and the Sipunculoids are nephrodinic. Too much importance must not be attached to the isolated observation of these testes, but it will be a matter of some significance if the ovaries should also prove to be saccular, and provided with a duct-like continuation of their own walls to the exterior.

It seems to me probable that the "cellular body" seen by Sars near the rectum may be a gonad. The position of the ripe testis, as now ascertained, is in favour of such an identification.

SOFT-STALK (*Gymnocaulus*), HARD-STALK (*Pectocaulus*),  
STALK-PIPE (*Caulotheca*), and BUD-FORMATION.

In describing the general form of the tubarium I have already indicated to a considerable extent the form of the colony of polypides which inhabits it. We have just reviewed the structure of a single polypide, with its long contractile stalk. How are the various polypides connected which together make up the colony? The answer is simple enough. They are connected by a branching stalk, which bears them upon it as an axis carrying appendages. This axial stalk branches from time to time. The axial stalk is essentially the same thing as the soft contractile stalk of a polypide. Every part of it has in its time actually been the soft contractile stalk of a polypide terminating a branch, and as the growth of the branch has advanced the soft stalk has become gradually shrunk to a narrow diameter, and has developed on its epidermis a hard, firm cuticle, of a very dark-brown colour. At the same time the cuticle, which thus forms a narrow black pipe around the shrunk stalk ("the stalk-pipe" or "*caulotheca*"), now rests against the floor of the tubarium, and becomes adherent to

and finally embedded in it. This is best seen in the transverse sections (Pl. XLI, figs. 9, 10, 11, *d, e, f*).

The gradual transformation of the soft stalk into the hard stalk by the development of a chitinous cuticle on its surface can be readily traced in every growing branch of a Rhabdopleura colony, and is seen in Pl. XXXIX, fig. 1, at the points marked *l, m, n*. The hard stalk is "the blastophore" or "chitinous rod" of Allman, from which Rhabdopleura derives its name. It does not appear from the writings of either Allman or Sars that they had ascertained the nature of this structure and its mode of formation. I should prefer to employ for it a name which expresses the fact that it is only the stalk of the leading polypide (for the time being) cuticularised and fixed to the wall of the tubarium, and accordingly whilst the contractile polypide-stalks are termed "soft-stalk" or "gymnocaulus," I would call the hardened portion "pectocaulus," and its cuticular investment the "stalk-pipe" or "caulotheca." The pectocaulus is no less living than the gymnocaulus; although no longer contractile the tissues within the stalk-pipe are in a living state, and serve as a vital connection between the different polypides. The fact that the pectocaulus is a hard pipe containing a soft medulla was established by Allman and confirmed by Sars.

A very interesting fact comes to light when the true nature of the pectocaulus is recognised, and this is that the chitinous stalk-pipe or caulotheca is the true homologue—the morphological equivalent—of the cœnœcium of an ordinary Polyzoan colony. This equivalence makes it all the more necessary to distinguish the tubular dwelling of Rhabdopleura by some other name, and justifies the special term "tubarium." The tubarium has no equivalent in Phylactolæmous and Gymnolæmous Polyzoa; the stalk-pipe or caulotheca is the cœnœcium of Rhabdopleura.

An examination of figs. 1 and 3 in Pl. XXXIX will now render the chief facts as to the bud-formation in a Rhabdopleura colony, and the building up of the tubarium and its transverse septa, intelligible. A branch of such a colony may

terminate in a fully-formed polypide, in which case we should be able to trace the gymnocalus of the colony back to the first septum of the tubarium, where its character would be found to suddenly change, and beyond the septum it would be shrunken and invested with a caulotheca and converted into pectocalus.

Supposing the branch to be a still-growing "proliferous branch," as was the case with most of the branches at the season of the year (August) when my specimens were obtained, we find that the tubarium of the branch-axis does not terminate in an upstanding polypide-tube, but is recumbent and fixed to the supporting surface up to its termination, which is not ring-like, but deeply notched (fig. 1, *a*, Pl. XXXIX). The terminal polypide is immature, having a very remarkable appearance. The buccal-disc is relatively of very great size, elongated in shape, and distinctly bifid. It is engaged in actively secreting the obliquely notched segments of the tubarium, the form of which depends on the form of the bifid buccal-disc. Placed superiorly to the elongated buccal-disc are the rudiments of the two lophophoral arms. The polypide-stalk (gymnocalus) follows close upon the elongated buccal-disc. It is not possible in this phase of the development of the polypide to distinguish thorax and abdomen. The polypide-stalk can be traced in the form of gymnocalus as far as a first transverse septum which is placed as a diaphragm across the tubarium (fig. 3, Pl. XXXIX); sometimes it may be traced through and beyond the first such septum to a second (fig. 1, Pl. XXXIX). Beyond either the first or second transverse septum the gymnocalus shows evidence of the formation of a heavy cuticle on its surface, and a little further back it is quite black (see for three stages of cuticularisation of the calus figs. 3, 4, 5, Pl. XLI).

The naked still-contractile part of the polypide-stalk shows one or more protuberances on its surface, which are very young buds. The youngest is that nearest the terminal polypide, which is itself in a rudimentary state. As we pass backwards along the stalk we find a series of buds, each one older than that in front of it, until we arrive at fully formed



polypides, which succeed one another in the same way as we pass further and further backwards along the stalk (see Pl. XXXIX, fig. 3, where the buds are numbered, and fig. 1). If we had a complete colony under study we should, in thus following back the stalk, come to its original starting-point, the point at which the embryo *Rhabdopleura* fixed itself when it began to assume the form of a polypide. Of this commencement I am not able to give any account; the oldest parts of the colony appear to have died down and broken away in all my specimens. Remarkably enough, the allied genus *Cephalodiscus* of McIntosh helps us to picture to ourselves the earliest condition of the *Rhabdopleura* colony, for in *Cephalodiscus* no colony is ever formed, but the polypide-stalk (about as long as its body) produces two buds near its base, which after attaining the form and a third the size of their parent, become detached. If the buds of *Cephalodiscus* were to remain attached to their parent, and did the parent's stalk elongate and continually produce new buds from its newly-grown region (that furthest from the original termination of the stalk) whilst the older region became cuticularised, we should have something like a *Rhabdopleura* colony without a tubarium. *Cephalodiscus* does not form a tubarium, but numerous detached individuals are sunk in cavities in a thick gelatinous substance, which forms a common investment to a vast number of separated free polypides, and has the same value morphologically as the tubarium of the *Rhabdopleura*.<sup>1</sup>

To return to the proliferous branch of *Rhabdopleura*. As soon as one of the very young wart-like buds has attained a certain size, a transverse septum of very delicate chitinous lamellæ is thrown down across the tubarium immediately behind it ( $p'$  in fig. 1, and just behind 3 in fig. 3, Pl. XXXIX). I am inclined to think that the septum is secreted by the epiderm of

<sup>1</sup> I am indebted to the kindness of Professor McIntosh and Mr. John Murray for the opportunity of examining the "Challenger" specimens of *Cephalodiscus*, which has already been described by the former naturalist, but will receive more ample treatment and illustration at his hands in one of the forthcoming "Challenger" Reports.

the young bud itself. Another septum will not be formed until the stalk has grown to a certain additional length. Thus in fig. 3, the stalk must elongate considerably between buds 2 and 3, and then a septum will be formed just behind bud 2; subsequently growth of the stalk will occur between buds 1 and 2, and then a septum will be produced behind bud 1. In the meantime the leading polypide-bud will have moved forward, forming new segments to the tubarium, and probably new rudiments of buds will have appeared between the present bud No. 1 and the leading bud. I am inclined to think that the leading polypide-bud does not advance so rapidly to its complete form as do the buds lower down the stalk, which are not called upon as this is to act as the leading growth of a proliferous branch. It seems likely (from the fact that this special form was often observed) that the leading bud is arrested in the condition shown in figs. 1 and 3 for a certain time, i.e. during the period of proliferation, and that only when its stalk ceases to be proliferous does the terminal bud go on to complete its own development. Apparently the completed polypide *f* in Pl. XXXIX, fig. 1, is actually of later origin than the leading immature polypide *b*; that is to say, it was formed as a bud on the stalk of this leading bud.

With regard to the formation of branches—that is to say, the acquirement of the bud-producing faculty by the portion of stalk supporting any given polypide, in virtue of which faculty the polypide-stalk at once becomes an axis or branch—I am inclined to think that it must be determined at an early period in the growth of the given polypide. I cannot find evidence of any complete polypide, here or there, in the colony, taking upon itself bud-production, after the complete development of the polypide. Such a thing may occur, but I have no evidence of it. It would appear that a branch is started by such a polypide-bud as No. 6 in fig. 3, Pl. XXXIX, breaking its way through the tubarium, and instead of itself developing and forming an upright tube, its stalk appropriates the nutriment, elongates disproportionately, and produces lateral buds. A branch may form at any point

on a mother branch; sometimes two or even three successive polypide-buds become proliferous, and then as a result we have the appearance of three or four branches diverging from an approximately common point.

In all cases the buds have to burst through the side of the chamber of the tubarium in which each is enclosed. The process has recently taken place in the instance of the polyp *e*, fig. 1, Pl. XXXIX. Upon the side of the axial tubarium thus ruptured the young polypide now constructs its own polypide-tube, at first recumbent and then upright. Thus it comes about that to every polypide there is an axial tubarium chamber, and a long tube opening into it at an angle.

Statoblasts or Hybernacula.—Allman has described what appear to be arrested buds which have not burst through the wall of the axial tubarian chamber as statoblasts. They appear to have been unusually numerous in Allman's specimen. It does not seem to be useful to apply the name "statoblast" to such arrested buds, because the true statoblasts of *Phylactolæma* have a totally different origin and position—are in fact produced within the body cavity, and have no relation to ordinary buds. The name "hybernacula," applied already to the arrested buds of *Paludicella*, would be more appropriate. I have come across several instances of such arrested buds in *Rhabdopleura*, but have no evidence to show that they will ultimately proceed to development, nor that their formation is due to anything more than an abnormality and distortion of the regular growth (Pl. XLI, fig. 6). Occasionally (as has been pointed out before) one finds not merely that a bud is arrested, but that it dies and decomposes without breaking its way out through the wall of the axial chamber of the tubarium (fig. 2, Pl. XLI). These "closed chambers," as Sars terms them, do not seem to have any regular distribution or functional significance. I should regard them as cases where the strength of the tubarian wall accidentally proved too much for the resources at the disposal of the young polypide in its attempt to break through; and very possibly the hybernacula are of similar origin. A remarkable instance of arrested buds

is drawn in fig. 2, Pl. XXXIX. The buds have almost the appearance of planula-larvæ. Possibly these are young Rhabdopleuræ developed from eggs and not buds.

#### AFFINITIES OF RHABDOPLEURA.

A knowledge of the development from the egg of Rhabdopleura should throw clear light on what is, in reference to this matter, now obscure and uncertain. Some years ago I advocated strongly the view that the buccal-shield or disc of Rhabdopleura and the epistome of the Phylactolæma is the equivalent of the molluscan foot. This view can no longer be maintained if we hold that Rhabdopleura is rightly classed with the Polyzoa, and that Phoronis also is to be classed with them. For Mr. Caldwell has shown that the epistome of Phoronis is the modified præoral hood of the actinotrocha larva, or at any rate its dorsal portion, and accordingly the epistome of Phylactolæma and the buccal-disc of Rhabdopleura and Cephalodiscus are DORSAL outgrowths. The molluscan foot is VENTRAL, and corresponds to the body and stalk of a polypide of Rhabdopleura (see my article, "Polyzoa," in the 'Encyclop. Britannica'). It is exceedingly probable that this is a true orientation of the three types Phoronis, Rhabdopleura, and Phylactolæma. But whilst it is a certainty so far as Phoronis is concerned, since we can identify in the minutest details the surfaces and ciliated areas of the Actinotrocha larva with the surfaces and areas of the trochosphere larva of Mollusca, there is no such certainty with reference either to Rhabdopleura or Phylactolæma; for of the first we do not know the larva, and of the second, as well as of all other Polyzoa, the larvæ appear to be secondary, non-recapitulative forms, which cannot be confidently referred to the trochosphere type, and consequently do not aid us in determining the relations of the regions of the adult to the dorsal, ventral, præoral, and postoral regions of a trochosphere. Hence we can at present only stumble along in the dark, in so far as Rhabdopleura is concerned. Whatever view is taken of the homology of the buccal-shield, it seems that there can be



no difficulty in referring the lophophoral arms and tentacles (as well as those of *Phoronis* and the *Eu-polyzoa*) to the same primary organ—the postoral portion of the architroch or ciliated band—from which the molluscan gill-plume is derived. Whilst the buccal-discs of *Rhabdopleura* and *Cephalodiscus* may possibly, after all, be shown by embryological fact to be ventral and not dorsal organs, it is well to remember that on the assumption that they are dorsal, we are not bound to conclude that they are necessarily prostomial, and correspond to the velar area of the trochosphere. On the contrary, they may represent (as may the epistome of *Phoronis*) a region nearer the anus, the prostomium being suppressed, as it is in *Lamellibranch* molluscs. Thus, then, Allman's suggestion, that the buccal-shield of *Rhabdopleura* represents the mantle area of *Lamellibranchia*, may prove to be correct.

The buccal-shield of *Rhabdopleura*, and still more so that of *Cephalodiscus*, is so peculiar that it is impossible to feel satisfied with any identification of it which does not rest on embryological grounds.

The fact that the buccal-shield secretes the tubarium, that in the bud the shield is very large relatively, and is more or less reflected over the small lophophor-rudiments and small polypide body, are in favour of identifying this organ with the *Lamellibranch's* mantle. Such an identification appears to involve the assumption that *Rhabdopleura* and *Cephalodiscus* are degraded *Lamellibranchs*.

All possibilities must be kept in view, and it is only when we have the embryology of *Rhabdopleura* that these questions can be decided. Even then it may possibly turn out that *Rhabdopleura* has a perverse kind of larva, which will defy the attempt to distinguish its trochospherical surfaces.

#### QUESTIONS FOR FUTURE OBSERVERS OF RHABDOPLEURA.

I will venture here to set down some of the points in regard to *Rhabdopleura*, concerning which further information seems especially desirable, and likely to be obtainable, if definitely

borne in mind, by those who may hereafter have the opportunity of studying *Rhabdopleura*.

1. Development of the first polypide of a colony from the egg. What regions of the polypide correspond to the velar (præoral) area of the trochosphere? What to its postoral area? What to its dorsal, what to its ventral surface?

2. Is the ovary idiodinic or nephrocinic?

3. Are the sexes distinct or united in one individual?

4. Do the colonies persist from one year to another? If so, do the polypides die down and leave only hybernacula and pectocaulus to continue the organism in the next season?

5. Do all the polypides develop sexual organs? Is there any special period of bud-production distinct from the period of sexual activity?

6. By what method does the young budded polypide make its way through the wall of the axial chamber of the tubarium in which it is at first closely shut?

## EXPLANATION OF PLATES XXXVII *bis*, XXXVIII, XXXIX, XL, and XLI,

Illustrating Professor Ray Lankester's memoir on "*Rhabdopleura*."

### PLATE XXXVII *bis*.

FIG. 1.—Complete colony of *Rhabdopleura Normani*, Allman. Drawn from a specimen found growing on the test of *Phallusia mammillata* at Lervik, Stordoe, Norway. Magnified about three times linear. The test of the Ascidian is represented of a uniform brown tint, the recumbent attached portions of the tubarium of the *Rhabdopleura* are also shown as brown, the upright polypide tubes are uncoloured. The black line within the tubarium represents the hard stalk (pectocaulus) and other portions of the zoarium, the polypides being all completely retracted. *a*. Growing ends of branches of the tubarium. *b*. The oldest portion of the tubarium. *c* (in Fig. 2). Bifurcation of a growing branch. *d*. Completed branches terminating in an upright polypide tube.

FIG. 2.—A similar colony more richly branched.

## PLATE XXXVIII.

FIG. 1.—A single polypide of *Rhabdopleura Normani*, Allman, as seen when issuing from the mouth of its tube. Highly magnified. The tube, which would reach up to about the level of the letter B, is not represented. *A*. Mouth. *B*. Anus. *C*. Polypide stalk (contractile stalk, soft stalk, or gymnocaulus). *D*. The buccal-disc or shield (præoral lobe). *E*. The intestine, showing through the rounded posterior projection of the "abdomen." *F*. The richly-pigmented thorax. *G*. A gill-filament or ciliated tentacle of the left tentaculiferous (lophophor) arm. *I*. The transparent abdomen, the yellow colour due to the gastric epithelium showing through.

FIG. 2.—A lateral view of a similar polypide. The left tentaculiferous arm is incompletely drawn for the sake of clearness. Letters as in Fig. 1, with the addition of *K*. Sars' ciliated sense (?) organ. *Ga*. Axis of the right tentaculiferous arm.

FIG. 3.—A similar polypide seen from the anal aspect, the tentaculiferous arms incompletely drawn. Letters as in Figs. 1 and 2, with the addition of *Dp*. Pigment spot of the buccal-disc (probably a rudimentary eye).

FIGS. 4, 5, 6, and 7 are intended to show different positions and forms assumed by the buccal-disc. In Fig. 1 its flat surface is thrown upwards, in Fig. 2 the disc is bent on itself so as to make the flat surface concave. In Fig. 4 the disc is elongated, and the pigmented extremity stretched forward; in Fig. 5 it has contracted to a nearly pentagonal form; Fig. 6 gives a view from the oral aspect of the position shown in profile in Fig. 2; Fig. 7 gives a profile view corresponding to Fig. 5.

FIG. 7.—The letters are as follows:—*D*. The broad or flat surface of the buccal-disc. *G*. Gill-filament (in outline). *H*. Raised margin of the tentaculiferous arm, where it joins the circum-oral region of the thorax. *Ga*. Axis of the tentaculiferous arm.

FIG. 8.—Extremity of a gill-filament or tentacle, to show the disposition of the pigment in the epithelial cells.

## PLATE XXXIX.

FIG. 1.—A portion of a colony of *Rhabdopleura Normani*, Allman. Highly magnified. Comprising a gemmiferous or growing branch, *a* (to the left), and six complete polypides with their tubarium. *a*. Extremity of the growing branch, showing the deeply-notched form of the growing margin of the tubarium, due to the bifid form of the foot of the immature polypide *g*, by which the segments of this part of the tubarium are secreted. *b*. Soft stalk or gymnocaulus of the terminal polypide of the growing branch. *c*. Youngest or latest bud produced by the gymnocaulus. *d*. Penultimate bud, that part of the stalk which produced it having now ceased to be "gymno-

caulus" and become "hardened stalk" or "pectocaulus." *e*. Third bud: it has reached the stage when it must force its way through the wall of the axial tubarium, and is commencing to construct a lateral polypide tube (*e e*) for itself, ring by ring. *e e*. The commencement of the polypide tube of the polypide *e*. *f*. The fourth bud in age, reckoning back from the youngest. It has reached the condition of a fully-grown polypide, and has constructed a complete polypide tube with recumbent and vertical (*x*) portions. *g, h, i, k*. The fifth, sixth, seventh, and eighth polypides, budded from this axis. Between *f* and *g*, however, a proliferous bud has developed, giving rise to the branch *z*. The forking of the pectocaulus is hidden here by the stalk of the polypide *g*. *l*. Stalk of the proliferous polypide which terminates the branch *z*, and is at this point still in the condition of "gymnocaulus," or "soft stalk." *m*. Older portion of the stalk, in which the formation of a cuticular investment is commencing. *n*. Still older portion of the stalk, now in the condition of "pectocaulus" or "hard stalk," enclosed in a thick brown cuticular tube, and fixed to the floor of the tubarium. *o*. Small pedicle of pectocaulus supporting lateral polypides. *p*<sup>1</sup> to *p*<sup>9</sup>. Successively-formed septa, dividing the axial portion of the tubarium into a series of chambers, corresponding in age to the series of polypides the most recent being *p*<sup>1</sup> and the oldest *p*<sup>9</sup>. *q*. Buccal-disc of immature polypide, usually showing bilobate form. *r*. Tentaculiferous or lophophor-arms of polypide buds. *s*. Polypide stalk of buds. *ss*. Polypide stalk (gymnocaulus) of complete polypide. *t*. Tentaculiferous arms of complete polypide. *u*. Buccal-disc of complete polypide. *w*. Abdomen of complete polypide. *x*. Point at which the polypide tube ceases to be recumbent and fixed to its support (the Ascidian test, a dead shell or corallum), and commences to bend upwards towards the vertical position. *y*. The vertical portion of the polypide tube. *z*. Branch which is given off between the septa *p*<sup>5</sup> and *p*<sup>6</sup>, and equally with the branch *a*, is a continuation of the axis which bifurcates to form them.

FIG. 2.—Portion of the tubarium of *Rhabdopleura Normani*, which has grown in the form of a coil and contains six oval buds of an exceptional form. *a*. Pectocaulus. *b*. Gymnocaulus. *c*. Normal bud. *d*. The six peculiar buds, some apparently detached from the gymnocaulus.

FIG. 3.—Outline sketch of a proliferous branch of a colony of *Rh. Normani*, in order to show the form and size of the successive buds. The buds are numbered in order of age, No. 1 being the youngest. The leading bud or immature polypide is, although less advanced in development, older than any bud carried by the branch, older therefore than No. 7; as also in Fig. 1, the leading bud of the branch *a* is older than the fully-formed polypide *f*, which is the last on its branch. Letters as in Fig. 1.

FIG. 4.—Portion of a polypide's tube, to show the way in which the component rings interlock.

FIG. 5.—Portion of the axial tubarium, to show internal upgrowths *a a*, which form an incomplete tube within the tubarium, overlying the pectocaulus.



## PLATE XL.

FIG. 1.—Portion of one of the tentaculiferous arms of *Rhabdopleura Normani* denuded of its soft tissues, except at the extremities of two tentacles (*c*), in order to expose the cartilaginous skeleton of the axis and filaments. *a*. Axis. *b*. Gill-filaments or tentacles. *c*. Soft tissues still adherent to extremity of two tentacles.

FIG. 2.—Diagram of a portion of the same skeleton.

FIG. 3.—Base of the skeleton of a tentacle, after addition of acetic acid.

FIG. 4.—Skeleton of the free end of a tentacle, after acetic acid.

FIG. 5.—A bit of the skeletal cord of the polypide stalk. *a*. Showing the same structure as the skeleton of the tentacles. *b*. Showing fusiform muscular fibre-cells on one side of the cord.

FIG. 6.—A portion of the polypide stalk of a fresh specimen, showing the skeletal cord within, broken up (by pressure?) into transverse discs. *a*. Outer epithelium of the stalk. *b*. Skeletal cord.

FIG. 7.—A polypide seen from the left side, the arms incompletely rendered. The specimen here drawn was one of seven observed, which were provided with a well-developed testis. *a*. Buccal-disc. *b*. Tentaculiferous arms. *c*. Intestine. *d*. Polypide's stalk. *e*. Anus. *f*. Testicular sac or testis. *g*. External orifice of the same near the anus.

FIG. 8.—Ripe motile spermatozoa, discharged from the testicular sac of the animal drawn in Fig. 7.

FIG. 9.—Testicular sac of another individual.

FIG. 10.—Blind end of a third testicular sac, showing corpuscles *a*, which appear to be sperm-blastophor cells.

FIG. 11.—Diagram showing the disposition of the skeletal elements in a polypide of *Rhabdopleura*. *a*. Buccal-disc. *b*. Skeleton of the arms. *c*. Its continuation around the thorax. *d*. Skeleton of the stalk. *e*. Anus.

FIG. 12.—Drawn from a living specimen of *Rhabdopleura* under the compressorium, to show the body cavity. *c*. Polypide stalk. *g. c.* Gastric cavity. *b. c.* The body cavity or coelom. *e.* Aboral region of abdomen. *ax.* Axial skeletal cord of the stalk. *ct.* Connective (mesoblastic) tissue cells. *eps.* Deric epithelium. *epg.* Enteric epithelium.

## PLATE XLI.

FIG. 1.—Pigmented epidermic cells, in natural grouping, from the polypide stalk of *Rhabdopleura Normani*. *a*. A brown-coloured cell in course of dissolution. *b*. A similar black cell.

FIG. 2.—An unoccupied or "permanently closed" chamber, *B*, of the axial tubarium, with portions of two others, *A* and *C*. *d*. The pectocaulus travers-

ing the floor of the untenanted chamber without giving off any bud. *e*. Purple-coloured material, such as is often seen occupying an empty chamber, and probably due to the breaking down of the tissues of a bud or polypide enclosed in the unopened chamber.

FIG. 3.—Portion of the stalk from such a branch as that drawn in Pl. XXXIX, fig. 3, taken about the region *m*, showing the gradual conversion of the soft-walled brilliantly-coloured gymnocaulus into the hard dark-brown pectocaulus. A thin cuticle, the commencement of the caulotheca or stalk-pipe, already invests the portion of stalk here drawn, but the coloured epidermal cells show through it.

FIG. 4.—A further stage in the conversion of the gymnocaulus into pectocaulus.

FIG. 5.—Completion of the change. The caulotheca is now so dense that the pigment spots of the enclosed stalk are no longer visible. The stalk has shrunk much in bulk, and the stalk-pipe (caulotheca) has shrunk and hardened at the same time, and is now embedded in laminæ of the tubarium (see Figs. 9 and 10).

FIG. 6.—Portion of the axial tubarium, showing a small closed chamber containing a spherical bud (*g*) apparently in healthy condition (? a hybernaculum). *a*, *b*, *c*.—Successive chambers of the tubarium. *d*. Pectocaulus. *e*. Wall of the tubarium in optical section. *f*. Stalk of a polypide not further shown in the drawing. *g*. The spherical bud, or hybernaculum. *h*. Grains of silex enclosed in the tubarium. *N.B.*—In this and Fig. 7 the laminar structure of the septa of the tubarium are well seen.

FIG. 7.—Three polypides, *c*, *e*, *g* (of which the last is still a very young bud), given off nearly simultaneously from the axis *xy*. The polypide stalk (gymnocaulus) of the polypides *e* and *f* are supported each on an unusually long pedicle of pectocaulus, *h*<sup>1</sup> and *h*<sup>2</sup>. Septa divide the chambers *a* from *b*, and *b* from *c*, *d*, and *e*.

FIG. 8.—Gastric epithelium of *Rh. Normani*.

FIG. 9.—Transverse section across the axis of a colony of *Rhabdopleura Normani*. Owing to the strong contraction of its stalk a polypide has been withdrawn into the axial chamber, and is seen in section. *A*. Test of the Ascidian, to which the tubarium is adherent. *B*. Substance of the tubarium (coloured yellow). *c*. Cavity of the axial chamber. *d*. Caulotheca, stalk-pipe, or cuticular investment of the pectocaulus embedded in the substance of the tubarium. *e*. Cavity of the pectocaulus, due either to shrinking of the soft tissue away from the cuticular investment, or possibly a normal space between the pectocaulus and its sheath. *f*. Soft substance of the pectocaulus, showing pigmented epidermic cells. *g*. Epidermic pigmented cell. *h*. Intestine. *i*. Stomach. *k*. Muscular tissue of body wall. *l*. Thoracic origin of the axial skeletal cord of the polypide stalk. *m*. Body cavity (cœlom).

FIG. 10.—Transverse section further along the same axis. The whole area

of the tubarium is not represented. A polypide stalk (gymnocaulus) is seen lying freely in the cavity of the tubarium, whilst two pectocauli are embedded in its floor (due to bifurcation and formation of two branches, or else to the pedicle of a polypide). Letters as in Fig. 9, with the addition of *x*, polypide stalk in transverse section.

FIG. 11.—Transverse section of same axis as in Fig. 9, at a point a little posterior to that given in Fig. 9. The hinder part of the abdomen of the polypide is cut a little obliquely and transversely so as to avoid the intestine, whilst the polypide stalk is seen as a separate piece. Letters as in Fig. 9.

FIG. 12.—A section of the same polypide as that seen in Fig. 9, taken at a point in front of that section. *m*. Body cavity. *o*. The thick columnar epithelium of the thoracic region. *p*. Mesoblastic cells traversing the body cavity.

FIG. 13.—A section between Figs. 9 and 13. *h*. Intestine. *i*. Stomach. *m*. Body cavity. *p*. Mesoblastic cells.

*N.B.*—Figs. 12 and 13 should be turned upside down for comparison with Fig. 9.

## Caldwell's Automatic Microtome.

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With Plate XLII.

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THIS machine has been devised to save labour to the histologist by cutting a very great number of sections suitable for microscopic investigation in a very short time. The machine is worked by hand, and may easily be made to deliver in one continuous band accurately cut sections at the rate of 100 per minute. Much higher numbers than this are obtained in the Cambridge laboratory, where during the past year one machine has done all the work for a large class of students. Not only are all the sections of equal thickness, but they are arranged in consecutive order and are all the same side upwards, which avoids a great loss of time and risk of fracture in the mounting. To use it, however, to the best advantage, it is well to drive it by means of some motor, the flywheel being already provided with a groove for the reception of the cord coming from the motor. Where there is sufficient pressure and supply of water, a simple form of water motor seems the most appropriate and least expensive.

### METHOD OF USING THE MICROTOME.

Place one of the cylindrical vessels supplied with the machine upon a piece of paper on a glass plate, and pour into it sufficient melted paraffin to fill it. As this cools the paraffin will contract and will leave a hole, which must be filled up with more melted paraffin.

Melt a small quantity, say an ounce, of embedding material in some suitable vessel; a small copper pan or a porcelain



crucible answers very well, if care is taken not to allow it to become hotter than is sufficient to thoroughly melt it. Take a piece of glass and smear it with a very small quantity of glycerine, to prevent the embedding material from sticking to it. Then pour the melted material on the glass, in small quantities at a time, so as to get a layer nearly a quarter of an inch thick. This when cut up into suitable pieces with a knife does very well for embedding small objects. If larger objects are required it is well to have two pieces of brass of the form shown in fig. 5, which, when placed together, will form a cavity half an inch in depth, and of any desired length up to an inch or more; this cavity may be filled with the melted material in the manner as already described, and the object to be cut must then be placed in position while the material is fluid. It is well to cool the material as rapidly as possible by placing it in water as soon as it is sufficiently set. From the cake thus formed, or from the piece cast in the mould, cut the piece of the material containing the object, and with an old scalpel, heated in a Bunsen flame, melt a small hole in the paraffin containing the cylindrical vessel (fig. 1, *a*), and insert the piece of embedding material containing the embedded object; then with the heated scalpel melt a little of the paraffin round the base of the projecting piece, so as to give it firm support, and allow this to become thoroughly set.

Now remove the large brass plate from the top of the microtome (fig. 1, *b*), and insert the vessel containing the embedded object in the tube for its reception, having first oiled the tube slightly to prevent the vessel from sticking. Next with a sharp knife cut the material with the object embedded in it, so that all its opposite sides are parallel. This is extremely important. Replace the top plate and fix the razor in the holder provided for the purpose. The clamp is so made that if a little care is taken the edge of the razor will not be injured (fig. 6). The razor must be set so that its back is as high as possible, as shown in fig. 6, and above all the razor must be extremely sharp. It should be sharpened on a stone and not on a strop. The sharpness of the razor and the accurate

parallelism of the sides of the mass to be cut are the most important points in the whole process.<sup>1</sup> Underneath the frame containing the object is a large brass milled head (fig. 2, *c*). By turning this the object may be raised or lowered according to the direction in which it is turned. This should be done until the object is just below the edge of the razor. The plate holding the razor should then be moved so that the edge of the razor is close to and quite parallel with the mass of material to be cut<sup>2</sup> (fig. 3). The plate should then be clamped by the screws at each side of it. A few turns of the flywheel will now bring the razor in contact with the object to be cut. The band of black ribbon (figs. 1 and 3, *d*) is now to be placed so that the end of it should be just above the razor and clamped in that position. When the handle is turned the sections should come off the razor in the form of a ribbon.

The ribbon of sections will not find its way to the continuous black band without assistance. With a needle in a handle or with the point of a scalpel pick up the end of the ribbon, when a sufficient length of it has been cut, and place it on the black continuous band, up which it will travel. When it reaches the top of the band suitable lengths may be cut off with a pair of scissors. It may be found that the black band travels either too slowly or too fast. Its speed may be varied by moving the ring (fig. 1, *e*) up or down upon the vertical

<sup>1</sup> The Cambridge Scientific Instrument Company, Cambridge, the makers of the instrument, have nearly completed an automatic machine for sharpening razors, since it has occurred to them that this is an operation which may be performed with much greater accuracy by mechanical means than by hand.

<sup>2</sup> The distance through which the sliding stage moves can be altered by raising or lowering the arm (figs. 1 and 4, *j*). This distance should be so arranged that the surface of the embedding mass containing the object to be cut just clears the razor when the sliding carriage is at its maximum and minimum distance from either end of the machine. This is important as the speed with which the black band travels varies directly with the throw of the machine. If this adjustment is made and a little care is used in adjusting the ring (fig. 1, *e*), see below, the ribbon will move at each turn of the flywheel through a distance equal to the breadth of the surface which is being cut. If on the other hand the object swings far beyond the razor the band will travel too quickly and probably break the string of sections.

brass arm: upwards, if it is moving too fast, downwards if too slow. A frequent cause of failure in the proper movement of the band is that the ebonite roller at the bottom of it is allowed to press against the razor. This must be avoided.

Varying the thickness of the sections.—In fig. 2 will be seen a milled head *f*, which when turned controls the movement of the clicks which acting upon the ratchet-wheel attached to the micrometer screw regulate the thickness of the sections. This may be done so as to allow the clicks to engage one half, one, or several teeth of the ratchet-wheel as may be required. When arranged for one half tooth, the sections will be  $\frac{1}{16000}$ th of an inch (.0025 mm.) in thickness, when arranged to engage a whole tooth  $\frac{1}{32000}$ th of an inch (.005 mm.), and so on. At first it is well to use a whole tooth, as when thinner sections are cut so much depends on the sharpness of the razor. After cutting for some time, the machine will suddenly stop, the object ceasing to rise when the handle is turned. This means that the full extent of the micrometer screw has been reached. It is necessary then to turn the large milled head (fig. 2, *c*) downwards, which will allow the carriage containing the object to fall to its lowest limit. It will be necessary now to raise the socket (fig. 2, *g*) in which the object is held so as to be in position to come in contact with the razor. This milled head (fig. 2, *c*) is useful for rapidly getting the object in proper position and avoiding considerable loss of time in turning the handle. The frame (fig. 2, *h*), which holds the socket, is arranged with two quadrants, so that the socket may be set at any angle desired, and may be clamped with the milled head underneath it. This is for use when the object has not been symmetrically embedded. The nut (fig. 1, *i*) is for tightening up the spring which draws the carriage of the machine back after having been pulled forward. In case this does not work properly, it is only necessary to unloose the two screws and, with some strong but blunt pieces of steel placed in the two holes, to rotate the nut so as to give a proper tension to the spiral spring. When this is done, the screws should be tightened up again to keep the nut in place.

The lock nuts (figs. 1 and 2, *s*) should be screwed up sufficiently tight to barely prevent the carriage from falling by its own weight, so that when the milled head (fig. 2, *c*) is screwed down a slight pressure with the finger is necessary to make the carriage fall.

To arrange the machine for cutting different sized blocks of material, it is only necessary to raise or lower the arm (figs. 1 and 4, *j*). When this arm is in a vertical position, the machine is arranged for its maximum traverse. When turned to the right and placed horizontally, it is at its minimum traverse. The cord, however, must always be in the groove of the wheel *k*.

It is important to keep the strings which give motion to the endless band in proper position. The string (fig. 1, *l*) should go from the end of the wire *m* round the groove *n* in the pulley and thence to the elastic band *o*. The elastic band *o* should be stretched and placed over the hook attached to the arm *p*, care being taken that the shorter end of the arm *p* is uppermost. The string *q* should be tied to the stud upon which the arm *p* is supported, going thence round the groove *r* of the pulley, and back again to the hook at the longer and lower end of the arm *p*, to which it should be tied.

**Embedding Material.**—The ribbon method of cutting sections depends very much upon the temperature of the room in which the operation is performed, since any given sample of paraffin will only cut within a small range of temperature. To meet this difficulty suitable embedding material has been produced, so that sections may be satisfactorily cut within a very considerable range of temperature, obviating the necessity of exactly adjusting the temperature of the room to the specimen of paraffin in use, or as an alternative, of providing a number of specimens of paraffin with different melting points.

**Method of Preparing the Slide.**—Make by the aid of heat a viscid solution of white shellac in light-coloured creosote. Spread a smooth, thin, and even layer of this solution on a clean dry slide, with a camel hair brush or with the little finger. Arrange the ribbon containing the sections on this



slide while moist, and place it in the dry shelf of the water bath, which should be at a temperature slightly above the melting point of the embedding material used. It should be left here until the creosote has evaporated and the embedding material melted. Now allow the slide to cool, and then wash it with turpentine until all the embedding material is dissolved. Canada balsam in chloroform or turpentine and the cover slip may now be applied in the usual manner. For convenience of mounting, it is extremely important that the ribbon of sections should be quite straight, and in order to ensure this it is necessary that the sides of the embedding material from which the sections are cut should be quite parallel. The straight ribbon, when obtained, should be removed to some clean surface and there cut into lengths appropriate to the size of the cover slips used. It will be found convenient to use cover slips at least two inches long: indeed a useful length for slides and cover slips is six inches for the former and four inches for the latter.

A Method of Embedding the Specimen to be Cut.—After the specimen has been stained it should be left in 90 per cent. alcohol for a few minutes, and thence transferred to absolute alcohol, there to remain until all the water is extracted. The length of time necessary for this varies greatly with the size of the specimen. A three-day chick, for instance, will require about an hour, larger specimens a day or more, in which case the absolute alcohol should be changed occasionally. Some tissues may be transferred directly from the absolute alcohol to turpentine, and thence in about two hours to the melted embedding material. For delicate tissues, however, the following process, though longer and more troublesome, is greatly preferable. With a pipette, introduce some chloroform to which two or three drops of ether have been added, under the alcohol in which the object is lying. The object will then float for some time at the junction of the alcohol and chloroform, and will finally sink into the chloroform when saturated with it. If, as often happens in the case of embryonic tissues, the object is lighter than the chloroform, it is not easy to tell when the saturation is complete, but generally on shaking the

bottle a saturated tissue can be temporarily covered by the chloroform, while tissues containing alcohol keep steadily on the surface.

When the tissue is saturated with the etherised chloroform it should be transferred to pure chloroform and there left for a few minutes. Then drop in some pellets of soft paraffin and leave it for two hours or more, shaking occasionally. The whole should then be poured into a small melting pot and a quantity of embedding material added. The melting pot should then be placed in the water bath at a temperature of about 60° C., and there left until all the chloroform has evaporated, which may be determined by the absence of smell of chloroform on shaking. If much embedding material is required this process takes a day or two; it is therefore better, when the solution of embedding material is fairly strong, to take out the tissue and put it direct into pure melted embedding material. In any case no chloroform must remain in the material to be cut, as it makes it brittle. Generally speaking the more gradually these processes are passed through the better will be the result.

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## On the Arrangement of the Embryonic Membranes in Marsupial Animals.

By

**H. Caldwell, B.A.,**

Fellow of Caius College, and Balfour Student of the University of Cambridge.

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With Plate XLIII.

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THE facts to be mentioned in the following short notice have hitherto been not only imperfectly but erroneously described.

Since my arrival in Australia I have obtained considerable numbers of early embryos of various marsupial animals.

Of *Phascolarctos cinereus* ("Native Bear") I have a very complete series of nearly 100 embryos in all stages from the unsegmented ovum onwards, while of the various kangaroo and wallaby types I have embryos in most of the stages. I shall not deal with the history of the germinal layers or of the general development in the present paper. The descriptions which follow holds especially for *Phascolarctos cinereus* and *Halmaturus ruficollis*.

These species represent two of the main divisions of the Marsupials, viz. (i) the short-faced opossum-like, and (ii) the long-faced kangaroo-like forms. I think it fair to regard the condition found in these as the typical marsupial one.

Both *Phascolarctos* and *Halmaturus* breed twice in each year, producing a single young one on each occasion. Exceptions to this are by no means uncommon, and in one "native bear" I found three blastodermic vesicles in one uterus.

**Ovum.**—The ovum arrives in the uterus from the Fallopian tube at a very early stage. In one instance I found an unsegmented ovum in the uterus.

**Uterus.**—Both uteri are very much enlarged in the early stages, and up to the time when the blastodermic vesicle measures about 8 mm. in diameter, the unoccupied is equal in size to the occupied uterus. The size of the uterus during gestation is due to the enormous development of its inner lining. The muscular coat also becomes slightly thicker. This inner lining is composed of a number of coiled tubular glands. Each gland is a hollow tube with large secreting cells forming the wall. Each gland opens separately by a small pore into the cavity of the uterus. I believe that the function of these glands is to supply a nutritive fluid for the growth of the young embryo. There is no vascular connection developed in any stages of the development between the embryo and the uterine wall.

The blastodermic vesicle lies quite unattached in the uterus. The zona radiata persists until the vesicle attains a diameter of 15 mm.

**Subzonal membrane.**—Meanwhile the amnion forms in the ordinary way. The false amnion continuous with the ectoderm covering the blastodermic vesicle I shall speak of as subzonal membrane.

While the embryo is being folded off from the yolk-sac another process is going on at the same time.

**Yolk-sac.**—The yolk-sac grows round the embryo in the same manner that the amniotic folds did in an earlier stage. This may be also described by saying that the embryo sinks into an indentation in the side of the yolk-sac, which finally forms an almost complete investment similar to the amnion and closely overlying that membrane. The walls of these yolk-sac folds, however, do not meet. They leave a circular area where the amnion is only separated by fluid from the subzonal membrane.

**Allantois.**—Into this circular area the allantois grows, and in the last period of intra-uterine life completely fills up the



space. In this way the embryo comes to hang in the interior of a vesicle whose outer wall is the subzonal membrane. The vesicle is oval and attains a size of 35 mm. in its longest, and 25 mm. in its shortest diameter. The yolk-sac occupies the greater part of the inner surface of the subzonal membrane.

Vascular area of yolk-sac.—The allantois in its greatest development occupies an area of about 12 mm. diameter, and in the later stages becomes vascular, but never develops villi. Professor Owen long ago describes the main features of the vascular supply of both yolk-sac and allantois. The whole vascular area is covered by flat cells of the subzonal membrane. There is no attachment to the uterus in this region.

Attachment of embryo to uterus.—The large oval vesicle, with the embryo suspended in its centre after attaining a diameter of 12.5 mm., begins to attach itself to the wall of the uterus. This attachment is caused by the growth of the cells of the subzonal membrane immediately outside the sinus terminalis; the cells of the subzonal membrane begin to enlarge and become amœboid. They throw out pseudopodia-like process, which fit in between the cells of the uterine epithelium and serve to attach the blastodermic vesicle to the uterus. This attachment is entirely non-vascular, and is the sole means by which the vesicle is attached to the uterus.

Just before birth the vesicle is sharply marked out into two areas, one of which has a smooth glistening surface and corresponds to an area of flat epithelium covering the vascular area of the yolk-sac and the vascular allantois; the other has a white opaque appearance and corresponds to the area of large amœboid attaching-cells. In *Phascolarctos cinereus* the attached area is next the opening of the uterus into the vaginal passages. In the kangaroo-like forms the attached area occupies that part of the uterine wall next the opening of the Fallopian tubes into the uterus.

Last year I was too late to obtain the early stages of either *Ornithorhynchus* or *Echidna*, still I think it probable from the structure of the uterine wall and from the appearance of a

membrane which I found in the uterus of an *Ornithorhynchus* whose young had just been born, that the Monotremes possess a somewhat similar arrangement to the condition described above in *Didelphia*.

Before this is published I shall have obtained the early stages of *Ornithorhynchus*, but I have decided to send home this communication without waiting, because of the interest which attaches to the discovery of the exact relations of the embryo to the maternal parts in these Mammalia.

The facts above described, so far as I see at present, throw little light on the evolution of the placenta in *Monodelphia*. The arrangement in the *Didelphia* is a unique one. I shall defer the discussion of it until my future papers on the development of the embryo itself.

## DESCRIPTION OF PLATE XLIII,

Illustrating Mr. H. Caldwell's Paper "On the Arrangement of the Embryonic Membranes in Marsupial Animals."

FIG. 1.—Advanced embryo of *Phascolarctos cinereus*, removed from the uterus by slight maceration to show the relation of the embryo to its membranes. Natural size. *s. z.* Subzonal membrane. *am.* Amnion. *al.* Allantois. *y. s.* Yolk-sac (umbilical vesicle). *s. t.* Sinus terminalis. The mesoblast forming the vascular area of the yolk-sac and allantois is indicated by the red line.

FIG. 2.—Section through a portion of the wall of an advanced vesicle of *Halmaturus ruficollis* in the region of the sinus terminalis, showing the transition from the flat cells of the subzonal membrane covering the vascular area to the amœboid attaching cells covering the non-vascular area of the yolk-sac. *a.* Subzonal membrane. *b.* Hypoblast of yolk-sac. *s. t.* Sinus terminalis. *c.* Blood-vessels. *p.* Blood-corpuscles. *amb.* Amœboid cells, with processes torn out of uterine epithelium. *f.* Flat cells. Zeiss, oc. 2, Obj. D.

**On the Fate of the Blastopore and the Presence  
of a Primitive Streak in the Newt (*Triton  
cristatus*).**

By

**Alice Johnson,**

Demonstrator of Biology, Newnham College, Cambridge.

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With Plate XLIV.

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THE coincidence of the blastopore with the anus in the Newt has already been observed by Mr. Sedgwick.<sup>1</sup> His assertion was, he says, based only upon surface views. He therefore suggested to me that I should attempt to verify it by cutting sections of the embryos, and my results confirm what he has stated.<sup>2</sup>

**I. THE FATE OF THE BLASTOPORE.**

A.—At the close of segmentation the blastopore is placed in the normal position at the hind end of the embryo. With the greater growth of the dorsal surface, consequent on the appearance of the medullary folds and formation of the medullary canal, it comes to occupy a place on the ventral surface at some distance from the hind end (vide fig. 11). Its distance from the hind end increases as development goes on (vide figs. 12, 13). The tail

<sup>1</sup> A. Sedgwick, "On the Origin of Metameric Segmentation and some other Morphological Questions," this Journal, January, 1884.

<sup>2</sup> Some of the main points of this paper have already appeared in a communication made to the Royal Society in June, 1884.

begins to bud out behind it, at a time when about ten mesoblastic somites have been formed, as a small conical knob whose blunt apex points forwards, and the tail has become very distinct in the stage represented in fig. 13, when there are about eighteen somites, and the rudiments of the sense organs, cerebral vesicles, visceral arches, &c., have appeared.

The blastopore leads into the hind gut, whose cavity is here broad, but very shallow (vide fig. 8). At a greater distance from the blastopore the cavity becomes much narrower and no deeper (vide fig. 6), so that it is very difficult to follow it in transverse sections. In the longitudinal sections, however, its continuity is quite apparent (vide figs. 11, 12, 13). Fig. 14 represents a transverse section, showing the open blastopore at a time before the tail is formed, and figs. 15, 16, 17, a series of transverse sections, showing the passage of the blastopore into the hind gut at a considerably later stage with a very distinct tail.

I find no stage at which the blastopore is closed.

B. Historical. — Scott and Osborn<sup>1</sup> describe a posterior dilatation of the medullary canal, the sinus rhomboidalis, which remains open for some time after the rest of the canal is closed. They say that its folds enclose the blastopore, and, therefore, when they come together, a neurenteric canal is formed. Their account of the exact date of the closure of the sinus rhomboidalis is a little obscure, but seems to indicate that it takes place while the number of mesoblastic somites is quite small, and before the rudiments of the visceral arches and of the tail have appeared.

Hertwig<sup>2</sup> figures an open blastopore at a slightly later stage than this, but he describes it as being situated at the end of a small conical process, which, judging from his surface views of the embryo, one would take to be the tail.

<sup>1</sup> W. B. Scott and H. F. Osborn, "On the Early Development of the Common Newt," this Journal, October, 1879.

<sup>2</sup> O. Hertwig, 'Die Entwicklung des Mittleren Keimblattes der Wirbelthiere,' Jena, 1881.



Bambeke<sup>1</sup> states that the blastopore disappears before the formation of the medullary folds.

As to the fate of the blastopore in other Amphibia, I conclude from Clarke's<sup>2</sup> account of the development of *Amblystoma* that it becomes the anus in this form, though the fact is not actually expressed in so many words. He says (p. 7), "At the extreme anal end the (medullary) folds remain separate over a small area, the space formerly occupied by the vitelline plug (the mass of yolk-cells which projects into the cavity of the blastopore and nearly fills it up at an earlier stage), and form a rounded edge about this small cavity or pit" (p. 8). "In a ventral view . . . are seen both the optic vesicles . . . and the anus at the posterior end of the neural tube" (p. 9). "The beginning of the tail also shows distinctly, and its median ridge, at the end of which is the dark cavity of the anus, is now much increased in size." No mention is made of the closure of the blastopore, and Clarke's figures (pl. ii, figs. 9, 10, 12, 14) confirm my deduction.

In *Pelobates*, Bambeke<sup>3</sup> states that the anus appears to him to correspond to the place formerly occupied by the "bouchon de Ecker" (vitelline plug). He figures it at a comparatively early stage (vide plate iv, fig. 5).

## II. THE PRIMITIVE STREAK.

A.—The first structure to appear on the surface of the ovum after the segmentation has been completed is a groove which generally extends from the blastopore along the greater part of the dorsal surface. This is the "Rückenrinne" of the German observers, the "Sillon médian" or "Sillon primitif."

<sup>1</sup> Ch. van Bambeke, "Nouvelles Recherches sur l'embryologie des Batraciens," 'Archives de Biologie,' vol. i, 1880.

<sup>2</sup> S. F. Clarke, "Development of *Amblystoma Punctatum*," part i, external, 'Studies from the Biological Laboratory of the Johns Hopkins University,' No. 2, 1880.

<sup>3</sup> Ch. van Bambeke, "Recherches sur le Développement du *Pelobate brun*," 'Mémoires Couronnés, &c., de l'Acad. Roy. de Belgique,' 1868.

tive" of Bambeke, and may obviously be called the primitive groove. Hertwig<sup>1</sup> says that it is at all stages sharply marked off from the blastopore by an intervening ridge of cells. In my specimens this sometimes occurs, but it happens at least as frequently that the groove is continuous with the blastopore at its first appearance, and I always find them continuous after the formation of the medullary folds.

Transverse sections through an embryo with a primitive groove and before the medullary folds have been formed shows that in the region of the groove the three embryonic layers are continuous with one another (vide fig. 1, which represents a section taken through about the middle of the embryo). It happened in this embryo that the primitive groove was continuous with the blastopore. In the anterior part of the embryo the groove flattened out and gradually disappeared. Fig. 2 represents a section through the groove near its anterior end, and shows that here the epiblast is distinct from the other two layers, the mesoblast still retaining its connection with the hypoblast. The mesoblast has generally been described (viz. by Scott and Osborn, Hertwig, and Bambeke<sup>2</sup>) as being derived exclusively from the hypoblast, except at the blastopore, from the lips of which it grows. It appears to me, on the contrary, that the greater part of it is derived from the primitive streak as in the higher Vertebrates, for it is seen in fig. 2 that the mesoblast cells, where they are represented as derived from the hypoblast, are much fewer in number than appears in fig. 1, where they are shown growing out from the primitive streak.

The primitive groove in another embryo of a slightly later stage exhibits a deep pit at its anterior end. I am unable at present to state whether any fusion of the layers exists in the region of this pit at this time.

The next step forwards in development consists in the for-

<sup>1</sup> O. Hertwig, loc. cit.

<sup>2</sup> Ch. van Bambeke, "Formation des feuillets embryonnaires et de la Noto-corde chez les Urodèles," 'Bulletins de l'Acad. Roy. de Belgique,' 2me série, tome 1, 1880.

mation of the medullary folds. In fig. 2 the dorsal half of the epiblast is seen to be thickened. This occurs first in the anterior part of the body, where also the folds are first clearly formed. They consist of a pair of sharply-marked ridges, bounding a very wide, flat area. The medullary plate, which includes the whole of the dorsal surface, is made up of narrow deep columnar cells. The rest of the epiblast, which formerly consisted of a single layer of columnar cells (vide figs. 1, 2), now begins to divide into two layers of flatter cells. These well-known peculiarities of the medullary plate and general epiblast have already been sufficiently figured by previous observers.

The primitive groove at this period extends from the blastopore throughout the whole medullary plate. The consequent division of the latter into two halves is especially conspicuous in front. It occasionally happens that the groove is absent in the middle region of the body. This was the case with the embryo, transverse sections of which are represented in figs. 3, 4, 5, and in which the medullary folds existed anteriorly, but diminished gradually and vanished behind. Fig. 3 shows the open blastopore, with the three embryonic layers coalescing at its edges in the ordinary manner. In fig. 4 the rounded primitive groove is seen indenting the primitive streak. In fig. 5 the groove is flatter, but the layers are still fused beneath it. The blastopore itself at this stage is narrow and elongated.

In another specimen of the same stage as that just described I find that the primitive groove extends for a short distance in front of the medullary folds. Near its anterior end it becomes rather suddenly considerably deeper and also loses its rounded outline, being instead triangular in section and sharply pointed at its apex. It presents in this region, in fact, an appearance strikingly similar to that of the blastopore, although not communicating with the archenteron. I believe, however, that the epiblast and hypoblast are fused at this point, and it can hardly be doubted that this deep pit, with the fused layers at its apex, represents the front end of the blastopore. It is evidently the same structure as the pit found at the front end

of the primitive groove at an earlier stage, and corresponds in position more or less with the future mouth.

As the medullary folds approach one another the primitive groove becomes gradually obliterated in the narrowing and folding up of the medullary plate, and the primitive streak remains only in the hind region. At the front end of this reduced primitive streak the sides of the medullary plate come together to form a solid mass instead of the thick-walled canal that exists in front. This fact is illustrated in figs. 6, 7, 8, and 9, which are taken from a series of transverse sections through the hind end of an embryo. The medullary canal in passing back round the hind end gradually loses its lumen (vide fig. 6, where the medullary canal is seen above and the solid mass of epiblast cells below). Further forwards on the ventral surface this solid mass becomes fused with the underlying hypoblast cells and the lateral plates of mesoblast. The primitive streak, thus constituted, forms a slightly pronounced ridge on the surface of the embryo (vide fig. 7). Nearer the blastopore the ridge is flatter (vide fig. 8). In fig. 9 the blastopore itself is seen with the continuity of the layers at its lips.

Fig. 10 shows the primitive streak, as seen in transverse section, of an embryo with a distinct tail, rudiments of the visceral clefts, &c. In figs. 11, 12, and 13 the primitive streak of different stages is shown in longitudinal section, but it cannot then be distinguished so clearly.

I have been unable to find at any stage the neurenteric canal mentioned by Scott and Osborn.

In the course of the development, the medullary canal is gradually differentiated backwards out of the primitive streak, and the hind gut, from being curved as seen in fig. 13, becomes straight.

The arrangement of the layers in the primitive streak of the Newt at the stage represented in figs. 6—9 resembles closely that described by Professor Balfour in the tail of the embryo *Lepidosteus*.<sup>1</sup>

<sup>1</sup> F. M. Balfour and W. N. Parker, "On the Structure and Development of *Lepidosteus*," 'Phil. Trans. of the Roy. Soc.,' part ii, 1882.



B. Historical.—The great breadth and flatness of the medullary plate at its first appearance is a well-known characteristic of Amphibian embryos. They are further distinguished at this period from the embryos of other Vertebrates by the division of the medullary plate into two symmetrical halves by means of the dorsal or primitive groove. This feature, as well as the continuity of the primitive groove with the blastopore, has been noticed by almost all observers of Amphibian embryology. Hertwig<sup>1</sup> alone denies the continuity of the two structures, but it has been described by Bambeke<sup>2</sup> in Triton and Axolotl; by Clarke<sup>3</sup> in Amblystoma; by Ecker<sup>4</sup> in the Frog; by Götte<sup>5</sup> in Bombinator; and by Bambeke<sup>6</sup> in Pelobates. Prévost and Dumas<sup>7</sup> figure the primitive groove in the middle of the medullary plate of the Frog, but do not mention its blastopore.

The only other Vertebrate, as far as I know, in which a similar disproportionately broad medullary plate and a like relation of the primitive groove to the medullary plate and blastopore have been described, is the Sturgeon. Kowalevsky, Owsjannikoff, and Wagner<sup>8</sup> describe in the embryo Sturgeon a specially broad medullary plate, in the middle of which is an opaque streak, which they call the "Primitivstreif," though they do not assert that any fusion of the layers exists there. A "Primitivrinne" runs down the centre of the "Primitivstreif," ending in the blastopore (vide their figures on pp.

<sup>1</sup> O. Hertwig, loc. cit.

<sup>2</sup> Ch. van Bambeke, "Nouvelles Recherches, &c.," loc. cit.

<sup>3</sup> S. F. Clarke, loc. cit.

<sup>4</sup> A. Ecker, "Icones Physiolog.," 1851—1859.

<sup>5</sup> A. Götte, "Die Entwicklungsgeschichte der Unke," Leipzig, 1875.

<sup>6</sup> Ch. van Bambeke, "Recherches sur le développement du Pélobate brun," loc. cit.

<sup>7</sup> Prévost and Dumas, "Deuxième mém. s. l. génération. Développement de l'œuf des Batraciens," 'Ann. Sci. Nat.,' ii, 1824.

<sup>8</sup> A. Kowalevsky, Ph. Owsjannikoff, and N. Wagner, "Die Entwicklung d. Störe," 'Vorläuf. Mittheilung. Mélanges Biologiques tirés du Bulletin de l'Acad.,' Imp., St. Pétersbourg, vol. vii, 1870.

175, 176). In Salensky's<sup>1</sup> account, however, no such structure as a primitive groove is mentioned or figured.

The solid condition of the hind end of the medullary canal, such as I find in the Newt, has been described by Strahl<sup>2</sup> for the Lizard, and by Gasser<sup>3</sup> for the Bird.

### III. SUMMARY OF FACTS AND GENERAL CONSIDERATIONS.

In the Newt (1) the anus of Rusconi, or blastopore, becomes the actual anus of the adult.

(2) A primitive streak exists on the dorsal surface in front of the open blastopore.

(3) The primitive groove extends along the whole of the dorsal surface from the open blastopore, and for a short distance in front of the medullary folds.

(4) The front end of the primitive groove deepens into a distinct pit, at the apex of which there is, almost certainly, a fusion between the hypoblast and epiblast.

The Newt affords another instance of the variability of position of the last open part of the blastopore in different groups of the Chordata.

In *Amphioxus*, the blastopore is posterior, and gives rise to a neurenteric canal on the formation of the medullary folds and closure of the medullary canal.

The same is the case with the *Ascidians*. In *Elasmobranchs*, the blastopore is converted into a neurenteric canal on the closure of the medullary folds. Behind this, there is a yolk blastopore, which closes without leaving a trace.

No neurenteric canal is known in *Teleosteans*, and an invagination, giving rise to a blastopore, has not been described.

<sup>1</sup> W. Salensky, "Recherches sur le développement du Sterlet," 'Archives de Biologie,' vol. ii, 1881.

<sup>2</sup> H. Strahl, "Beiträge zur Entwicklung von *Lacerta agilis*," 'Arch. f. Anat. u. Phys.,' 1882.

<sup>3</sup> Gasser, "Der Primitivstreifen bei *Vogelembryonen*," 'Schriften d. Gesell. zur Beförd. d. gesammten Naturwiss. zu Marburg,' vol. ii, supplement i, 1879.

In *Petromyzon*, an invagination takes place. The blastopore remains open for a long time, though not permanently. The medullary canal is formed first as a solid cord, which becomes continuous with the hypoblast at the lip of the blastopore, thus forming the rudiment of a neurenteric canal.

In *Acipenser*, the invagination blastopore is converted into a neurenteric canal.

In *Lepidosteus*, there is no open blastopore of the ordinary kind, formed by means of an invagination, but Professor Balfour says: "In the region of the tail, the axial part of the hypoblast, the notochord and the neural cord fuse together, and the fused part so formed is the homologue of the neurenteric canal of other types. Quite at the hinder end of the embryo, the mesoblastic plates cease to be separable from the axial structures between them" ('Comp. Embryology, vol. ii, p. 93). This arrangement seems to be comparable with the primitive streak and neurenteric canal at its front end, such as is found in the higher Chordata.

In Amphibians generally, the invagination blastopore gives rise to a neurenteric canal. In the Newt, however, the invagination blastopore becomes the anus. A primitive streak extends along the dorsal surface in front of the blastopore, and I believe that there is no neurenteric canal. The primitive groove, which extends in front of the medullary folds, has a deep pit at its anterior end. In *Amblystoma* also, as mentioned above, the blastopore probably becomes the anus.

In Reptiles, there is an invagination blastopore, which becomes a neurenteric canal. Behind this point there is a primitive streak. The anus is formed along the line of the primitive streak, which extends at least as far forwards as the opening of the allantois into the alimentary canal in the Lizard,<sup>1</sup> and probably in all types having an allantois. Strahl<sup>2</sup> states that in the Lizard, the invagination begins in the middle of the primitive streak, near, but not at, its front end. By the

<sup>1</sup> W. F. R. Weldon, "Note on the Early Development of *Lacerta muralis*," this Journal, January, 1883.

<sup>2</sup> H. Strahl, loc. cit.

time that the hypoblast has been perforated by the invagination, the differentiation of the layers has extended as far back as the blastopore. Therefore, when a neurenteric canal is formed, this exists at the front end of the now reduced primitive streak.

In Birds, the invagination blastopore occurs comparatively late in development, e. g. it is most fully developed in the Duck with twenty-six mesoblastic somites and a medullary canal closed except at the extreme hind end. A neurenteric canal is found at a later stage. The primitive streak exists only behind the invagination blastopore and its corresponding structure, the neurenteric canal. At the latter, the hypoblast is fused with the epiblast and mesoblast, but remains separate from these two layers throughout the rest of the primitive streak.

In Mammals, the invagination blastopore begins as a pit in the epiblast at the front end of the primitive streak.<sup>1</sup> It then extends downwards and perforates the blastoderm completely. When the medullary groove is formed, it constitutes a neurenteric canal, piercing the floor of the hind end of the groove, but, before the medullary folds close, its ventral opening into the archenteron has become obliterated, and its upper part alone remains.

The view that the primitive streak represents part of the original blastopore is now so generally accepted that it may be assumed here for purposes of argument.

No reason has been suggested for the various behaviour of the blastopore of the Chordata in these different cases. It is sometimes a simple opening which gives rise to a neurenteric canal and then vanishes altogether. In other instances, it is elongated and composed of a primitive streak with an opening (which becomes a neurenteric canal) generally at its front end, but in one form (the Lizard) in the middle, or the opening may be (in the Newt) at the hind end of the primitive streak and persist without having any connection with a neur-

<sup>1</sup> W. Heape, "On the Germinal Layers and Early Development of the Mole," 'Proc. Roy. Soc.,' 1881.



enteric canal. Sometimes the blastopore merely consists of a primitive streak with no opening at all.

In all cases, except the Newt, the opening is restricted to the embryonic stages, and may be described as an embryonic structure. As to the variations, we can only say, for want of a more definite reason, that they are for purposes of embryonic convenience.

It is obvious, from all the facts adduced, that the original Vertebrate blastopore was elongated, but its present condition shows that great changes have taken place, since, even in the embryo, part or parts of the opening have been obliterated, these parts varying in different embryos. It is obvious, too, that the anus, at any rate, is derived from the original blastopore, and therefore is probably not an entirely new formation, acquired within the group, but is homologous with the anus of the primitive ancestral form.

My results show also that the primitive streak (i. e. blastopore) extends much further forwards than was supposed. In fact, the pit found at the front end of the primitive groove in the Newt corresponds in position more or less with the future mouth as has been remarked. This points to the probability of a connection between the blastopore and mouth, and so supports Mr. Sedgwick's<sup>1</sup> view that the blastopore of the Chordata was an elongated dorsal slit, the ends of which gave rise to the mouth and anus.

In *Peripatus*,<sup>2</sup> too, it is known that the blastopore is an elongated structure, the middle part of which closes, while the ends become respectively the mouth and anus of the adult.

The fusion of the embryonic layers is most distinct at the hind end of the embryo. I believe that it exists also at the front end of the primitive groove in the Newt. In the middle region of the body its existence is doubtful, but the fact that the primitive groove extends along the dorsal surface from the

<sup>1</sup> A. Sedgwick, loc. cit.

<sup>2</sup> F. M. Balfour, "Anatomy and Development of *Peripatus capensis*," this Journal, April, 1883.

open part of the blastopore to the anterior pit seems to prove that the blastopore as a whole is dorsal and not ventral.

A slight additional argument in favour of this view may perhaps be found in the much greater nearness of the archenteron to the dorsal surface than to the ventral in early stages of development before the yolk has been absorbed. It seems natural that the cavity should exist near the surface from which the involution to form it originally sprung.

It has already been mentioned that, in the Lizard, the primitive streak extends in front of the anus on the ventral surface as far as the opening of the allantois into the alimentary canal. Scott and Osborn described, at a comparatively late stage (with rudiments of external gills, &c.) in the Newt, a very distinct fusion of the hypoblast and epiblast in the middle ventral line behind the mouth. I have myself observed the fusion which they say is connected with the early formation of the thyroid body. Can this also be part of the primitive streak? If so, neither the mouth nor anus represent the extreme ends of the blastopore.

A possible connection between the two methods of formation of the mesoblast in Vertebrates, viz. as outgrowths from the primitive streak or lips of the blastopore, and as outgrowths from the hypoblast, is suggested by the theory of an elongated dorsal blastopore. We may suppose that, at a time when the blastopore was a long narrow open slit, the archenteron was a large cavity opening into it in the median line, and the mesoblast consisted of a pair of pouches opening into it on each side for its whole length. When the blastopore became closed and a separation between the epiblast and hypoblast ensued, the mesoblast naturally retained its connection with the latter, since it was functionally from the beginning an appendage of the archenteron. Of course, where the primitive streak existed the mesoblast would keep as far as possible traces of its original condition, but in regions where the primitive streak was obliterated the mesoblast could only proceed from the hypoblast.

In conclusion, I wish to express my very sincere thanks to

Mr. Sedgwick for his kindness in helping me both in my work and in the preparation of this paper.

## EXPLANATION OF PLATE XLIV.

Illustrating Miss Johnson's paper on "The Fate of the Blastopore and the Presence of a Primitive Streak in the Newt."

### *List of References.*

*a.* Archenteron. *au.* Auditory vesicle. *b. c.* Body-cavity. *bl.* Blastopore. *ch.* Notochord. *ch.'* Rudiment of notochord. *ep.* Epiblast. *ep'.* Thickened epiblast of medullary area. *f. b.* Fore-brain. *f. g.* Fore-gut. *h. g.* Hind-gut. *hy.* hypoblast. *m. c.* Medullary canal. *m. c'.* Solid medullary canal. *mes.* Mesoblast. *m. s.* Mesoblastic somite. *o. v.* Optic vesicle. *pr. g.* Primitive groove. *pr. s.* Primitive streak. *s.* Space between hypoblast and mesoblast. *v. c.* Outgrowth of fore-gut to form visceral cleft.

FIG. 1.—Transverse section through embryo before the formation of the medullary folds. The section is taken through about the middle of the embryo.

FIG. 2.—Transverse section through the same embryo, taken at some distance further forwards.

FIGS. 3, 4, 5.—Transverse sections through embryo in which the formation of the medullary folds has just taken place, and the medullary area is still very broad. Fig. 3 passes through the blastopore, and is the most posterior of the series. Fig. 4 is taken through the dorsal surface at some distance in front of the blastopore. Fig. 5 is the most anterior of the series near the front end of the posterior part of the primitive groove.

FIGS. 6, 7, 8, 9.—Transverse sections through an embryo with several mesoblast somites. Fig. 6 is the most posterior, and fig. 9 the most anterior of the series.

FIG. 10.—Section through the primitive streak of an older embryo, with rudiments of the visceral clefts, tail, &c. The section is oblique, i. e. between the transverse and horizontal planes, consequently the primitive streak appears deeper than usual.

FIG. 11.—Longitudinal section through the hind part of an embryo with the medullary folds just closed. The section is slightly oblique.

FIG. 12.—Longitudinal section through the hind part of an embryo with about twelve somites:

FIG. 13.—Longitudinal section through an embryo with about eighteen somites:

FIG. 14.—Transverse section through an embryo before the formation of the tail, showing the open blastopore.

FIGS. 15, 16, 17.—Transverse sections through a considerably older embryo than that of fig. 14, showing continuity of blastopore with hind-gut. Fig. 15 is the most posterior, and fig. 17 the most anterior of the series.

The light grey colour signifies the epiblast and organs derived from it, the dark grey signifies mesoblast, and the yellow signifies hypoblast and organs derived from it.

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## On the Eyes of Some Invertebrata.

By

**Justus Carrière,**

Of Strassburg.

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With Plate XLV.

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It is known that in the Vertebrata the percipient elements of the retina turn away from the light, while they are turned to it in the Invertebrata—an arrangement which is conditioned by the development of the organ in both the groups.

In order to attain the greatest effect and to embrace a very extended horizon the percipient elements can be arranged in two ways.

Either they form the wall of a hollow ball, into which the light falls through a very small opening, so that there is sketched a real image on the background of the eye. This kind of organ of sight is found in the vertebrates and the cephaloporous molluscs and lies below the surface of the body.

Or the parts of the eye perceiving the light are radially disposed on a more or less complete ball, which projects beyond the surface of the body. These eyes, forming a fan in vertical section, are met with among the Arthropoda and certain Lamellibranchiata.

A third form of eyes which belongs to the Arthropoda, the cup-shaped eyes with one lens, will not be considered in the present communication; nor will the organs of sight constructed upon the principle of the camera obscura be dealt with, since my purpose is merely to offer a few remarks in regard to fan-shaped (coniform) eyes.

Through Grenacher we know the construction of the eyes

(ommatidium) of *Musca*, while Berger first described the related layers of the optic ganglion. Already, some years ago, I myself examined both parts by means of thin vertical sections, and I can in the first place confirm Grenacher's accounts concerning the construction of the pseudoconus and of the retinula.

The whole set of eye-units (ommatidia<sup>1</sup>) of *Musca vomitoria* are enclosed in a chitinous capsule, the exterior face of which is formed of the cornea-lenses, while a thin chitinous membrane limits the eye towards the brain, being full of holes to let pass the nerve-fibres. It is only a little smaller than the cornea and nearly concentric to it. The eyes being placed on both sides of the head have their largest extension in the direction from above to below; the foremost retinulae stand almost perpendicular on the basal membrane; in the midst of the eye they take a position more inclined backwards, and the last ones are not only set quite slantwise, but even curved in order to catch still a glimpse backwards, while all the other retinulae are quite straight. With this setting of the eye-units the animal sees to the side, and obliquely to the front and behind, but not directly to the front. Experiments show also that a fly rests often quietly sitting if one approaches it with a fine-pointed object directly from the front; but upon approaching it from the side mostly makes off.

The eye is limited anteriorly by a fold in the membrane (Chitinpanzer)  $x$ , which is narrow and directed towards the inside, being specific to many insects.

Also on the dorsal margin such a fold, but less deep, is met with; whereas the posterior margin of the eye is supported by a round ledge ( $y$ ), the transverse section of which is shown by the drawing.

<sup>1</sup> The term "ommateum" was introduced by Lankester in his memoir on the eyes of Scorpions to signify the entire soft parts of the non-segregate (unicorneal) eye of Arachnida and Hexapoda, consisting of a layer of nerve-end cells, and usually a layer of vitreous cells. The similar term "ommatidium" is introduced in this paper to signify the units consisting each of a retinula and a vitrella, together with their sheath of pigment cells, into which the ommateum of the multicorneal (polymeniscous) eye of Arthropods, is segregated.

The side-wall (cornea) does not project very much over the surface of the head, and is divided into a great number of plano-convex lenses (facets) which are separated from one another by narrow shallow furrows.

I will here recall to mind quite shortly what Grenacher says about the construction of the eye-units :

Below each cornea-lens is found a coniform space (Pl. XLV, fig. 1, A) filled with a liquid or jelly ; its shape is apparently fixed by the enveloping pigment-cells. This is the pseudoconus. Its truncated point ends at the four crystal cells (4), which are also set together, so as to form a cone with its broad base outwards, the point inwards. They are the matrix-cells of the pseudoconus, and surrounded like it by the two principal pigment-cells (2).

The crystal cells are touched by the retinula (6), which diminishes in breadth as it passes inwards, and so represents a very much elongated cone, which surpasses the pseudoconus in length more than sixfold. The retinula consists of seven cells fused together longwise, and forming the wall of a tube, at the inside of which the staves (rhabdomeres) (5) project as circular ledges ; they touch each other only at the foremost somewhat thickened end. One of the rhabdomeres always lies in the midst of the six other ones carried by a narrow ledge of the cell-body.

Between the exterior ends of the ommatidia lie clear-coloured and spindle-shaped pigment-cells (3).

Of the seven nuclei of the retinula-cells five lie in the uppermost end of the retinula, while the sixth one lies somewhat more distant from them, and the seventh one quite isolated in the lower third of the retinula, so that one can distinguish clearly in a stained vertical section of the eye, three nucleus layers, the second and third of which, formed each by one row of nuclei, are placed concentrically to one another.

At the end of the retinula, immediately above the basal membrane, lie pigment-cells with very small nuclei (7), the numerous processes of which wrap round in a subtle pigment-knot the undermost part of the retinula.

Among the matrix-cells of the cribriform basal membrane, being there proportionately thick, numerous tracheæ ramify. The thicker trunks of these are placed between the matrix-cells and the ganglion layer lying below it.

No more about the optic and light-perceiving part of the eye. Behind it lies the ganglion apparatus which shows in *Musca* a typical, but, as it seems, not very frequent construction, the peripheral ganglion-opticum being spread out close to the eye as a plane.

Out of the central part of the brain passes a long and narrow string of nerve-fibres through an interposed ganglion layer into the central opticus-ganglion (13), radiating in it towards all sides.

This ganglion forms a cone turned with the point outwards, with the concave base inwards to the brain. The inside of the cone consists of several concentric layers of "Punktsubstanz" (to employ Leydig's expression), which are connected together by a great number of narrow granular strips, while the mantle of the cone is formed by the ganglion-cells or the nuclei of them.<sup>1</sup>

(The subtler construction of the ganglion can only be indicated in the Plate, because of the small size of the drawing, and has also been sufficiently illustrated by Berger, 'Arbeiten Zool. Anstalt. Wien,' vol. i.)

Through the cone-point consisting of ganglion-cells the nerve-fibres come forth rectilineally, and cross each other shortly after passing out as they keep their direction (12). After crossing they extend themselves again over a larger space, and at the same time the nerve-fibres always become connected in groups so as to form bundles. Between these cord-like bundles lie numerous nuclei of the most different form and size, probably appertaining to the connective tissue.

The exterior ganglion-opticum consists of a threefold layer of small ganglion-cells (9), and a single stratum of long pali-

<sup>1</sup> In the embryonic state perfect cells, with much cell-body, form the bark of the ganglion in insects; the cell-substance diminishes more and more in the course of the development, the deeper layers of the ganglion increasing probably at its expense.



sade-shaped cells (10), the number of which corresponds with that of the eye-units. Every one of these palisade cells possesses an oblong nucleus at its foremost somewhat broader end, and is broken into by one of the nerve-strings mentioned above.

With a *Sarcophaga carnaria* which I examined shortly before the creeping out of the pupa, and the eye of which was already quite developed and brown-pigmented, while the ganglion showed still somewhat the embryonal character, I could determine by means of transverse and vertical sections that the nerve-string in each palisade cell surrounds a refracting chitinous or cuticular tube which lies in the midst of the cell.

In *Musca vomitoria* also, one sees that in every cell lies a cylindrical axis, but on account of the small size of the histological elements and for want of transverse sections, I could not decide here if it be the nerve-string or such a chitinous tube. Also, I did not succeed in obtaining sureness as to whether the nerve-string perforates the palisade cell unaltered, or if it only passes close to it and suffers here an interruption by the substance contained in the tube or in the axial cylinder.

Inwardly the palisade layer is limited by a membrane (11) containing nuclei; on the outside a string of several nerve-fibres passes out of every cell, penetrates the layer of the small ganglion cells (9) and reaches the central end of the retinula. In vertical sections I could not of course see more than three or four fibres in one string, but it seems certain to me, in view of what occurs in other Arthropoda, that their number answers to that of the retinula cells, and probably that already the strings or bundles, formed after the crossing, consist each of seven fibres.

Though in most cases the peripheral ganglion-opticum is not extended as a plane but more spherically, yet there is a specific character which we always find in the higher and in many lower Arthropoda with fan-eyes—namely, the existence of the two ganglionic layers, and secondly, the crossing of the nerve-fibres between them. In the Decapoda and Schizopoda there

are actually four ganglion layers successively in the ommatophor and between each a crossing of fibres takes place.

---

If one considers the ordering of the eye-units in *Musca* as the one extreme of the form of the fan-shaped eye, the other is found in the Cladocera. The eye approaches here to the spherical form and exhibits its most developed form in *Leptodora hyalina*, where the spherical eye is carried freely at the front end of the body like a lantern on a thin stake.

In a section which bisects the eye in the median plane (Pl. XLV, fig. 2), there lie about twenty-four eye-units set radially; the strongly pigmented retinulæ lie quite closely to one another, so that in the transverse section the thin rhabdoms appear like clear points on a black ground.

The retinulæ do not reach with their points quite to the centre of the globe, but end so as to leave empty a little central space.

The crystal cones are slender and considerably longer than the retinulæ are composed of five segments and do not lie very closely together, and at the peripheral end show distinctly their limits (*a*) whilst they fuse more and more intimately together towards the cone-point (*b*, *c*, *d*). Their transverse section is accordingly here rosette-shaped, there circular.

At about the half height the cone shows a pyriform swelling which stains intensely with colouring reagents.

The crystal cone is surrounded throughout its length by a number of cells, the "Umhüllungsschlauch" (investing tunic) of Leydig; at the outermost end they form around the base of the cone a sack-shaped envelope hanging together; in which in one plane of section are visible four nuclei (*a*), so that their number may be estimated at five, and this part of the envelope with the nuclei may be regarded as the remains of the crystal cells (Semper's nucleus).

The thinner part of the cone is surrounded by long, narrow cells, the nuclei of which are somewhat irregular. I am not able to say if the nuclei which lie close round the cones before the beginning of the pigmented retinula (*d* and *b*) belong

to these cells or perhaps to the retinulæ, or if they are homologous with the pigment-cells.

The cone penetrates with its point deeply into the retinula. The latter surrounds a thin rhabdom which is probably four-edged.

The ganglion lies in front of the brain ganglion, separated from it only slightly by a narrow furrow. Its rind consists in the front and at the sides only of a thin, at the back of a thicker, layer of nuclei through which the nerve-fibres enter from the brain-ganglion into the ganglion-opticum. The medullary substance of the latter can be separated into a front and a back segment.

The outgoing nerve-fibres pass as a spindle-shaped string into the centre of the eye, where they touch the ends of the retinulæ. The string is without pigment until it subsides between the crystal cones, and is surrounded by a pigment envelope between the retinulæ.

With the exception of the entrance of the nerves, which occupies the space of about one or two eye-units, the eye-units radiate equally towards all directions, both forwards, sideways, and backwards, being directed to the translucent body.

---

Just as the camera obscura eye is common to the vertebrates and the molluscs, so there are also met with in the molluscs organs of sight of the type of the fan-eyes.

For a long time past eyes have been attributed to the Lamellibranchiata. If these animals possess such organs they can only lie on two places of the body: at the mantle border or on the siphon. At the latter place modern zoologists have not been able to find any organs of sight, whilst at the mantle border are situated, in many species, organs of different form and composition, which are considered partly wrongly (Pinna) partly rightly as eyes.

Their construction is the simplest in *Arca* and *Pectunculus*. In *Pectunculus glycymeris* there occur on the edge of the interior mantle fold small spherical dark-brown

pigment spots at a distance of about 0.5 mm. from one another. In *Arca Noae* they are black-brown, close crowded, at the back end of the mantle larger and more distant from one another, about thirteen in a length of 2 centimetres. These organs consist (Pl. XLV, figs. 3 and 4) of a small number of large cells of the form of an elongated cone, with the point directed inwards. From their lying close side by side results the outwardly convex form of the organ.

The pigment is found in the periphery of the cells, and surrounds as a sheath the cell body; the nucleus lies in the exterior half of each cell.

In *Pectunculus* the cuticular border is tolerably thick, and the exterior surface of every cell is convexly arched; the organ distinctly contrasts by its colour with the mantle fold, which is here without pigment.

The optic cells of *Arca* are larger, and the whole organ higher developed, for every cell possesses a kind of lens formed by its cuticular border, which is convexly arched, not only outwardly, but also inwardly. Since the pigment sheath reaches the exterior end of the cell this lens is only visible in cells, the pigment of which has been removed in the cutting of a section. Here the substance of this cuticular lens is distinguished plainly from the cell body by its power of refraction and its behaviour when treated with staining reagents.

The cells which form the organ of sense, the optic cells, are not limited sharply against the epithelial cells of the mantle, but pass quickly through long and narrow intermediate forms into the epithelial cells. Consequently the eyes of *Arca* and *Pectunculus*, as much as those of *Patella* and the Ocelli of the medusæ *Oceania* and *Aurelia aurita*, may be classed among the most beautiful examples for the study of the origin of organs of sight by the modification of epithelial cells.

Moreover, I think that these simple fan-shaped eyes of *Arca* and *Pectunculus* offer us a new proof of the truth of the view that similar organs of sight in different classes and orders of the animal kingdom may have originated independently, and in fact may still originate without in any way implying that



such eyes have been inherited from an ancestor common to these different classes and orders.

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## EXPLANATION OF PLATE XLV.

Illustrating Dr. Justus Carrière's Memoir "On the Eyes of Some Invertebrata."

FIG. 1.—Vertical section of the eye of *Musca vomitoria*. Stained with Haematoxylin. A. An ommatidium (eye-unit), more highly magnified. *c*. Cornea. *f*. Fold of the chitinous membrane. *g*. Ledge of chitin. 1. Pseudocoanus. 2. Pigment-cells (i). 3. Pigment-cells (ii). 4. Crystal-cells. 5. Rhabdomere. 6. Retinula. 7. Pigment-cells on the base of the retinula. 8. Basal membrane, with its matrix-cells. 9. Layer of little cells. 10. Layer of palisade cells of the peripheral ganglion-opticum. 11. Membrane. 12. Chiasma of nerve-fibres. 13. Central ganglion-opticum. 14. Intermediate ganglion.

FIG. 2.—A vertical section through the midst of the eye of *Leptodora hyalina*. B. An ommatidium of it, more highly magnified. *a*—*d*. Transverse sections through the crystal cone. *a*. Near the base. *b*. In the first third. *c*. In the second third. *d*. Close before the retinula. *g*. Ganglion-opticum.

FIG. 3.—Vertical section of the eye of *Pectunculus glycimeris*. 1. Epithelial cells. 2. Optic cell (nerve-end cell). 3. Connective tissue and nerve-fibres. 4. Transverse section of three nerve-end cells, in two of them the nucleus.

FIG. 4.—Vertical section of the eye of *Arca Noae*. 1. Epithelial cells. 2. Nerve-end cell, tangential section. 3. Nerve-end cell, median section. 4. Transverse section of a nerve-end cell. 5. Connective tissue and nerve-fibres.

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## Protoplasmic Movement and Quinine.

By

**Prof. Dr. E. Binz,**  
in Bonn.

IN No. xcv of this Journal, July, 1884, p. 396, the following sentence occurs in the translation of the treatise of Professor Engelmann in Utrecht from L. Hermann's 'Handbook of Physiology':

"Binz<sup>1</sup> and others have observed that quinine exercises a strongly destructive action on many kinds of protoplasm and on colourless blood-corpuscles. On the other hand, I have given frogs such large doses of quinine sulphate by subcutaneous injection as to kill them, and have observed the lymph-corpuscles after some hours in active movement."

My pupil, C. Scharrenbroich, now physician in Pallanza, has already published a reply to this statement of Engelmann.<sup>2</sup> He has shown that the discordant statement of Engelmann rests on two reasons: on a different manner of making the experiment I describe, and on a false interpretation of the stunted movements which Engelmann still saw in the colourless blood-corpuscles of his frogs. Professor Engelmann was good enough soon afterwards to write me as follows:

<sup>1</sup> C. Binz, "Ueber die Einwirkung des Chinin auf Protoplasmaabewegung," 'Arch. f. mikrosk. Anat.,' iii, p. 383, 1867.

<sup>2</sup> "Einiges Alte über Chinin," 'Arch. f. Pathol. u. Pharmakol.,' xii, p. 33, 1879.

“UTRECHT, *June 21st*, 1880.

“DEAR COLLEAGUE,—Allow me to add a few words to the dissertation of my pupil, C. ten Bosch, on Chinamine, which I sent to you to-day. As you will see from the work, in case the Dutch does not prevent you from reading it, I have made comparative researches with C. ten Bosch on the action of Chinamine and Quinine on elementary organisms, especially on white blood-corpuscles, and have thereby had opportunity to confirm your important results as to the extremely intense action of quinine on contractile blood-cells, also to verify the superiority of this body to Chinamine, which in other respects far exceeds the action of Quinine.

“I am glad thus to clear away a little mistake which I have caused through my over-sceptical remarks in L. Hermann’s ‘Handbook’ (art., “Protoplasmic Movement”). If I can render you a service thereby I will gladly publish a notice as to the settlement of this difference anywhere you think suitable, perhaps in continuation of Dr. Scharrenbroich’s article.

“Of course the remarks as to Quinine will be altered in a possible second edition of the first volume of Hermann’s ‘Handbook.’ The fact on which my sceptical remarks rest is beyond doubt, but it cannot form the basis of an objection to your statements.

I am, &c., yours,

“TH. W. ENGELMANN.”

“To Prof. Dr. BINZ, Bonn.”

It appears to me that the matter is thus settled. But should anyone wish for further confirmation of what I have published on the influence of quinine on protoplasm in the above quoted and in later papers, I would refer to the following :

(1) G. Kerner, ‘Arch. f. d. ges. Physiologie,’ iii, p. 136, tab. ii, v, p. 27 ; vii, p. 135, 1870—1873.

(2) Buchanan Baxter, ‘The Practitioner,’ a journal for therapeutics and public health, London, xi, p. 324, 1873.

(3) Ch. Darwin, 'Insectivorous Plants,' London, 1875, pp. 201—203.

(4) T. Appert, 'Arch. f. pathol. Anatomie,' lxxi, p. 364, 1877 (from Professor Arnold's laboratory, Heidelberg).

(5) C. Fr. W. Krukenberg, 'Vergleichend. physiolog. Studien,' Heidelberg, 1880, i, p. 8.

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